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The Design of Scaffolds for Use in Tissue Engineering

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Abstract

Problems: Tissue engineering (TE) is one of the modern strategies to provide a functional biological tissue equivalent to restore or improve the function of human tissues that lost by disease, traumatic events, or congenital abnormalities. Some essential factors in TE are tissue type and appropriate scaffold. suitable influence on cell viability and proliferation is One of the most important characteristics of a appropriate scaffold for tissue engineering. The purpose of this research was to comparative evaluation of adipose derived stem cells (ADSCs) proliferation rate and their viability on the five different scaffolds. **Experimental approach:** six different scaffolds were prepared including Alginate, Poly Lactic Glycolic Acid (PLGA), Fibrin glue (FG), Inactive Platelet- Rich Plasma (IPRP), Active Platelet- Rich Plasma (APRP) and Hydroxyapatite/ β-

tricalcium phosphate (HA/β-TCP). Human adipose tissue was applied as mesenchymal stem cells source. These cells were seeded in the targeted scaffolds. After 48 hours, the proliferation and viability of mesenchymal stem cells were assessed by MTT assay. The obtained results were analyzed statistically by SPSS software ($p < 0.05$). **Findings:** Significant differences between cell proliferation and viability of different scaffolds have been shown. Alginate and active PRP were shown to be the most and least suitable scaffolds in terms of enhancing cell proliferation and maintaining cell viability respectively ($p < 0.05$). **Conclusion:** Our study indicated that active PRP could be the best scaffold for support of Adipose-derived stem cells proliferation in comparison with others.

Key words: adipose-derived mesenchymal stem cells, MTT assay, alginate, PRP, PLGA, Fibrin Glue, Hydroxyapatite/ β-tricalcium phosphate (HA/β-TCP)

Introduction

Tissue engineering (TE) is one of the modern strategies to provide a functional biological tissue equivalent to restore or improve the function of human tissues that lost by disease, traumatic events, or congenital abnormalities. (1,2) Beside this main aim, it offers some additional benefits; the ex vivo systems provided by TE can be used to study human disease formation and therapeutic interventions, filling the gap between in vitro human cell researches and human clinical trials where currently animal models are used. (3) Although some TE approaches are based on cell-free materials and/or factors, but the standard approach of TE entail seeded of a three-dimensional (3D) biomaterial scaffold or carrier with cells which this construct can either be delivered to and retained within the damaged site or be pre-cultured to generate the needed tissue and later transplanted to the needed location. Thus, the choice of suitable cells and scaffolds are the major challenges in TE. (4, 5, 6, 7) To understand the importance of scaffolds, it is useful to consider studies that provide evidence of the ability of some biomaterial scaffolds to directly control stem cell differentiation. (8) In fact the choice of appropriate scaffold is very more complex than the cell type and depends on many factors. One of essential factors is the tissue type; the scaffolds for different TE applications should have distinct biological and

physical requirements. A number of other properties that have been shown to affect the eventual developed (resulted) tissue involve, but not restricted to chemical composition and architecture of scaffold, its porosity and pore size, hydro-phobicity and charge. PLGA is a type of synthetic polymer with the characteristics of biocompatibility, low immunogenicity and absolute biodegredablity and can be processed into any shape and size, and encapsulate molecules of virtually any size.(9,10,11) A fibrin scaffold is known as a network of protein that together and protected a different kinds of living tissues. Fibrin glues are mainly exploited from pooled plasma and consist of different amounts of purified and virally inactivated human proteins: Fibrinogen, thrombin, factor XIII, anti-fibrinolytic agents and calcium chloride. Fibrin scaffold can be used as a principal element in tissue engineering approaches and provide complete autologous construction.(12,13) Alginate is shown as a nontoxic hydrogel scaffold and hence has been used in tissue engineering for culturing of chondrocytes.(14) Other natural scaffolds, Platelet-rich plasma (PRP) are known as a new technology in treatment of various diseases. The first description of PRP was in the early 1990s, when science was focused on developing new "biological glues." PRP therapy is a safe alternative to surgery for mild to moderate tendon, ligament, and certain muscle injuries and often helps patients with decreased functionality and chronic pain, but who may not be candidates for surgery but offers benefits such as cast saving and shorter recovery time. Autologous (patient derived) Platelet Rich Plasma (PRP) Therapy is started more than 30 years for various medical purposes. (15-20) Nowadays, Hydroxyapatite (HA) is one of the most abundant bioceramics, Because of its important biochemical properties in mineral constituents of human teeth and bones. Also It is a biocompatible ceramics widely used in biomedical applications including tissue engineering. HA has ideal biocompatibility with hard tissues. (43, 39) However, it is also used in soft tissue engineering application such as cartilage. (21) Hydroxyapatite and Hydroxyapatite/β-tricalcium phosphate (HA/β-TCP) based bone graft materials are both "biocompatible" and "bioactive". Hopefully the results of this work and other similar work will lead to identification of better scaffold that has a major role in maintaining cells viable. Clearly the second step is to find correct scaffolds that can direct viable stem cells in wanted fate.

Materials and methods

Materials

PLGA 50/50 (poly lactic-co-glycolic acid) and alginate were purchased from Sigma. DMEM was purchased from Sigma and fetal bovine serum and penicillin-streptomycin were purchased from Gibco in order to preparation of cell culture medium.

Cell isolation and expansion of human stem cells

Adult stem cells were isolated from adipose tissue in liposuction surgery. Previously, consent was obtained from each patient. Briefly, the adipose tissue was enzymatically digested with collagenase I (Sigma) at 37°C for 45-60 min. After that, we centrifuged the isolated mesenchymal stem cells and resuspended them with DMEM supplemented with 10% fetal calf serum and 100U/ml penicillin/streptomycin. Then they were placed into cell culture flasks and cultured at 37˚C, 5% CO2 and 95% air. When reaching 90% confluence at passage 2, then ADSCs were harvested with 0.25 trypsin/ EDTA and were used for every one of scaffolds.

Preparation of PLGA scaffold

5% PLGA particles was dissolved in Methylene Chloride (CH2CL2) then mixed with NaCl. A plastic mold, 3mm in height and 6mm in diameter was then filled with the polymer/NaCl particles and frozen at -20˚C. The frozen polymer scaffold placed in distilled water for 2 days in order to remove the particles. Finally samples were sterilized by use of UV light. Cultivated ADSCs were trypsinized in the secondary passage (P2), and seeded into the scaffolds.

Preparation of alginate scaffold

To prepare alginate solution (1.2%), at first we dissolved alginate powder in 5 ml of %0.9 NaCl. Alginate solution was passed through 0.22 μm filter. After trypsinization, the cell-alginate mixture at cell densitiy of 5×10^6 cells/ml was dropped into 105 μm CaCl2 solution for 15 min. Then alginate beads were washed with NaCl solution and added DMEM. Ultimately they were placed at in an incubator (37°C, 5% CO2. 99% humidity and PH 7.4) for 48h. (21)

Preparation of fibrinogen and thrombin

Fresh frozen plasma (FFP) and fibrinogen were obtained from Blood Transfusion Organizations of Qom branch. Thrombin was separated by centrifugation of FFP at 2200 rpm for 10 min. In this way FFP was added to calcium gluconate 5:3 ratio and incubated at 37˚C for 1h. In the next level

supernatant was removed after centrifugation. Then fibrinogen and thrombin solution were ready for cell seeding. The isolated hADCs were suspended in thrombin at a density of 5×10^6 cells/ml and cryoprecipitation was added to them and then incubated in 37°C, 5% CO2 and 99% humidity for 48h.

Preparation of platelet-rich plasma (PRP)

PRP was prepared from peripheral blood into silicones tubes containing 3.8% sodium citrate at ratio 9:1 and subsequently were centrifuged at 1000 rpm for 5 min. The number of platelets was determined by a cell counter system. 3 distinct layers are visible in obtained product: the beneath layer consist of erythrocytes; the middle layer contains leukocytes and inflammatory cytokines; and the top layer content plasma, platelets and growth factors. Prepared PRP was divided to two groups: one activated by 10% solution of CaCl2 and the other was labled as inactivated PRP.

Preparation of Hydroxyapatite/ β-tricalcium phosphate (HA/β-TCP)

Hydroxyapatite/β-tricalcium phosphate (HA/β-TCP) block and cell seeding Hydroxyapatite/β-tricalcium phosphate block (ARTOSAL®) with dimensions of 1 mm× 4mm were purchased. The ceramic Block rinsed twice with distilled water . Each block was set in 4-well plate, and then 1 mL cell suspension was added to each well of 4-well plate and incubated for 48 h.

Cell viability using MTT assay

After 2 days (48h), MTT (3-(4, 5-dimethyl) thiazol-2-yl-2, 5-dimethyl tetrazolium bromide) assay was used for the cells viability and proliferation measurement. MTT solution (5mg/ml) was added to scaffolds/cells for 4h and then intra cellular formazan was solubilized by adding 1 mL dimethyl sulfoxide for 20min. Absorbance at 570 nm was measured on microplate reader.

Statistical Analysis

Viability of cells in different scaffolds was analyzed by use of SPSS software ($P < 0.05$).

Result

Cell viability was significantly different among scaffolds while in fibrin glue it was significantly lower than active PRP and more than alginate. The Cell viability was minimum in alginate and maximum in active PRP group ($P< 0.05$) (Table1 and Figure1). PLGA and HA/β-TCP had just significant difference with alginate ($p < 0.05$). finally, According to our results, between active PRP, inactive PRP, PLGA and control groups, no significant difference was observed

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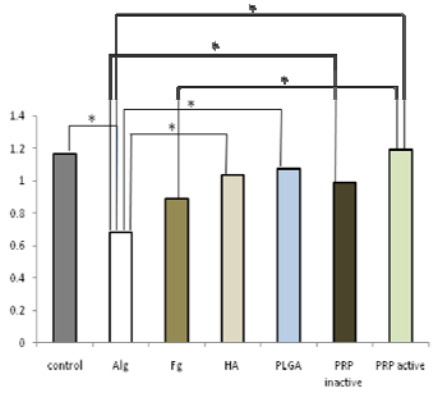

Figure 1- Viability of adipose-derived mesenchymal stem cells seeded on different scaffolds analyzed by MTT assay

Table 1- Comparison of mean ± standard deviation of proliferative activity of human Adipose Stem Cells Seeded in the different Scaffolds

Groups	Mean	Std. Deviation	minimum	maximum
Control	1.17	0.38	0.5	1.63
scaffolds				
PRP(active)	1.19	0.41	0.76	1.8
PLGA	1.07	0.2	0.74	1.42
PRP(inactive)	0.98	0.37	0.6	1.43
Fibrin Glue	0.88	0.21	0.53	1.15
Alginate	0.68	0.1	0.55	0.82
HA/β-TCP	1.032	0.2	0.81	1.35

Discussion

Stem cells present a great therapeutic potential due to their capacity of differentiation to many cell lineages. These cells are able to self renew and proliferate for long period in vitro (22, 23). Mesenchymal stem cells -in particular- present great potentials to the treatment of several diseases due to their low immunogenicity, immunomodulatory properties, possibility of autologous transplantation, easy isolation and in vitro proliferation possibility (24).

Already, the major source of stem cells for tissue engineering applications was bone marrow-derived stem cells but recently adipose-derived stem cells (ADSC) are attractive, abundant, and readily available cells that are used in many applications in tissue engineering [25,26].

Tissue engineering has been researched and developed to use in the repair or reconstruction of defective or injured organs. In tissue engineering, living cells by use of suitable scaffolds produce an artificial tissue. This chosen scaffold should provide the cells with the space for function and support their activities. Every scaffold has specific properties that fit some kind of cells or mimic

other tissues in mechanical strength, so we should regard all the characteristics of the target cells and tissues to choose the optimal scaffold (27). In this survey, we evaluated five scaffolds, considering cell viability and proliferation.

Active PRP in comparison with fibrin glue and alginate had the best effect on the cell proliferation and viability. Although alginate is used commonly as a scaffold in tissue engineering, but we showed lower ability of alginate in cell proliferation and viability. Active PRP was better than inactive PRP and PLGA scaffolds in the cell proliferation and viability but without significant difference.

Our result can be related to the advantages of PRP over other artificial or natural-derived scaffolds using in tissue engineering. PRP is defined as a sample of plasma with a twofold or more increase in platelet concentration above baseline level or greater than 1.1×106 platelets/μL (28).

Platelets physiological role in healing has led to the concept that PRP may improve cartilage restoration. Additionally, the multitude of growth factors stored within the platelets alpha granules are believed to improve the biological environment within which cartilage may heal (29). Multiple in vivo and in vitro studies are present in the literature delineating the potential of PRP to improve chondrogenesis in ankle cartilage repair (30, 31).

It is documented that bone-marrow derived mesenchymal stromal cells (BMSC) in PRP scaffolds are distributed properly (32, 33). Also it has been demonstrated that PRP acts as a powerful substitute of various growth factors in the culture of different kinds of cells, including mesenchymal stem cells, fibroblasts, dental stem cells and osteoblasts (34). Lee et al. showed an increase in growth and differentiation of PRP-treated periodontal ligament cells and stromal stem cells as type I collagen gene expression (35). Beside, the growth factors in PRP have a supportive role in cell migration, proliferation and differentiation, which all of them are critical for wound healing and soft-tissue augmentation. Hydrogel structure of PRP facilitate nutrition perfusion for pre-seeded and newly migrated cells and because of its plasticity and adhensive fixation to designated sites, it is so convenient. The other advantage of using PRP is that because every ones blood is used, there is no risk of transmissible infection, no side effects and a very low risk of

allergic reaction (36).

PLGA is a biopolymer that have been widely used as a common scaffold because of its distinct advantages like controllable biodegradation rate and mechanical properties, good biocompatibility and ease of processing into desired shapes (37). It is shown that PLGA nanofiber scaffolds accommodate the survival and proliferation of MSCs (37). Also by culturing composites of human bone marrow mesenchymal stem cells and porous PLGA scaffold tissue engineering of bone construct can be improved (38). However, the surface of synthetic polymers are hydrophobic, which limits cell adhesion and growth in 3D architecture (37).

As it is shown one of the important characteristics of fibrin is its increasing instability and solubility over time, due to fibrinolysis. Although it could be an advantage in wound healing or other surgical applications, when it is used as a shape-specific scaffold in tissue-engineering becomes a problem. Beside, cell adhesion is a problem in using these scaffolds. About 30-40% of the early seeded cells do not attach to the scaffold and their uses would be limited in future (39).

Alginate is a nontoxic hydrogel scaffold and hence it has been used in tissue engineering for culturing of chondrocytes. But one of the problems of alginate is that any significant interaction is not seen between mammalian cells and alginate gel (14).

Hydroxyapatite/ β-tricalcium phosphate (HA/β -TCP) composite scaffold has shown great potential for tissue engineering applications. In this study, we assess the potential of ceramic scaffold with different HA/ β-TCP compositions (pure HA, 60 HA/40 β- TCP, and 20 HA/80 β-TCP) as a scaffold in order to providing a suitable substrate for maintaining mesenchymal stem cells viability and causing their proliferation. Jiang et al revealed that β-TCP scaffolds are appropriate for mesenchymal stem cell survival and in vitro proliferation. Hashemibeni et al. have demonstrated an increase in growth of human osteoblast cells seeded on HA-TC scaffold more than monolayer culture, as well as obtained result of jiang et al expriement using β-TCP scaffold (5, 17 , 24). Also, Jalota et al have reported that cells were able to bind and proliferate well on the HA/ β -TCP composite (60HA/40 β - TCP) scaffold (16). By Using MTT assay, the HA- β – TCP Scaffold was proven to be a non-cytotoxic scaffold. beside, it would have Relatively suitable biocompatibility in vitro. Our results indicate that HA/β-TCP could be used as

suitable environment for growth and proliferation of adipose mesenchymal stem cells.

Therefore long term stability and mechanical integration of scaffold will be necessary for cells requiring enough stability and determined time for producing their tissue-specific matrix.

Conclusion

The obtained results show that active PRP can be consider as the best scaffold which supply a appropriate condition for adipose tissue derived stem cells that support their proliferation and viability in compare with the other four scaffolds(Alginate- inactive PRP- PLGA- Fibrin glue-HA/β-TCP). It seems that growth factors within PRP with their supportive role in cell proliferation can enhance its capability as a scaffold. On the other hand, Alginate was not as successful as the active PRP in supporting cell viability and proliferation.

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