PLATELET GEL: 3D SCAFFOLD FOR CELL CULTURE

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ABSTRACT

Introduction: Tissue repair has been the ultimate goal of surgery. Cell culture requires a mechanical scaffold that supports cell growth and nutrient diffusion. Using platelet-rich plasma (PRP) as a 3D scaffold presents various advantages: it is a biological material, easily absorbed after transplantation, rich in growth factors, in particular, PDGF-ββ and TGF-β that stimulate extracellular matrix synthesis in cartilage culture. Objective: To develop a PRP 3D scaffold. Material and Methods: Two forms were idealized: Sphere and Carpet. Sterile conditions were used. The platelet gel remained in culture conditions, observed at an inverted microscope on a daily basis. Results: Both forms were successful because they produced a 3D environment that supports cell growth, with positive and negative features. Discussion: The Sphere form didn't attach to the plate. Gel retraction was observed and the investigation at the microscope was difficult, because of the opaque areas in the optical field. The Carpet form didn't retract, and didn't produce opaque areas. Follow-up time was 20 days. Conclusions: The production of a PRP 3D scaffold was successful, and this is an alternative requiring further investigation in order to establish an efficient and reliable route in tissue engineering technology, particularly in cartilage tissue culture.

Keywords: *Cell culture techniques. Tissue scaffolds. Tissue engineering.*

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INTRODUCTION

The repair of tissues and organs has been the ultimate goal of surgery since the ancient times until present, and has traditionally been performed in two ways: tissue grafting or replacement by a synthetic material. Gold has been used in skull defects since 2000 B.C., and tissue grafting at least since 1660 A.C. Both methods, however, have limitations. Tissue grafting requires more surgical procedures associated to greater morbidity, and is restricted by a limited amount of materials. Synthetic materials integrate poorly with tissues and can fail with time due to wear off process and fatigue, or because of an adverse response of the body. Tissues engineering (TissEng)emerged in 1990 to fix the limitations of tissue grafting and synthetic materials use. This TissEng concept is to transplant a biofactor (cells, genes or proteins) into a porous degradable material (scaffold). Biofactors include stem cell and genetic therapy, which stimulate tissue repair.[1]

The purpose of tissues engineering is to restore and preserve the functions lost by sick or damaged organs. As what happens in vivo, projected tissues in vitro should provide nutrients, transportation, mechanical stability, coordination of multiple cellular processes and a cellular microenvironment that preserves the phenotypic stability of cells. In order to accomplish this, many projected tissues require architectural characteristics of macro scale (cm) and micro scale (approximately 100 microns) and cell culture techniques have been adjusted to create scaffolds with three-dimensional architectures defined in relevant scales to the physiological tissue success.[2] Otherwise, if cultured as monolayers, cells tend to adhere to the bottom of the container, and undergo a dedifferentiation process, when they acquire morphological characteristics and start producing elements of other cell types' matrix.[3] Thus, tissue function is lost, as seen in cartilage culture in monolayers, where chondrocytes dedifferentiate into fibroblasts, starting to produce type-I collagen.

Far from being passive components, the selected material and the porous design of the scaffold architecture are crucial factors for successful tissue regeneration. A successful scaffold should provide mechanical support while promoting massive transportation, also degrading for the regenerated tissue to assume its functions.[1]

Several culture systems have been employed: culture in monolayers, culture in agarose gel[4], hydrogel and synthetic polymers such as collagen-based scaffolds (type I and II collagen gel), type II collagen sponges, polylactic acid and polyglycolic acid, fibrin, polyethylene oxide, peptides and alginate[5], and, more recently, bacterial cellulosis.[6]

By investigating the role of platelets, its use as a gel was considered for serving as a 3-D scaffold for cell culture based on the release of a number of hormones making an interface with adherence, healing and neovascularization of its granules. The potential use of platelet-rich plasma (PRP) as a 3-D gel scaffold for cell support has several

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advantages. Among these, the biological nature of this material, which demonstrates an easy reabsorption ability after transplantation phase, its potential to be produced with a platelet concentrate withdrawn from the patient who will receive the transplant, the low cost of the material, in addition of its abundance of growth factors, particularly PDGF and TGF, which stimulate extracellular matrix synthesis[7], its importance for differentiation into various cell strains, especially in cartilage culture, and they are very expensive to acquire, as well.

OBJECTIVES

The challenge proposed by this study was to define the use of PRP as a 3-D scaffold for cell culture concerning its morphology, reagent concentrations and determination of the maximum number of days in which the gel remains stable in the presence of a culture medium.

MATERIALS AND METHODS

Five New Zealand rabbits have been used in this study, which was approved by the Committee of Ethics on Animal Experimentation of the institution, according to protocol nr. 626. All procedures were conducted in sterile environment. PRP was obtained by centrifuging total blood (withdrawn by heart puncture) at 1000 rpm for 10 minutes. After centrifugation, PRP was divided into aliquots in tapered 15-ml tubes and a sample (200 μl) was taken to determine the number of platelets in an automatic counter model Micros60 HoribaABX®. Once separated into 2.3 ml volumes, gels were prepared using a fixed strength of calcium gluconate and various thrombin strengths on Petri plates. The gels were tested with fresh and unfrozen PRP.

Two forms were suggested for gels: a thicker one, and a more uniform and thinner carpet (but still in 3-D). These forms were obtained as follows: once calcium gluconate (CG) and thrombin were added to PRP in the tapered 15-ml tube, solidification was expected for placing the gel on the Petri plate, thereby obtaining the Sphere gel, or, either, soon after CG and thrombin were added to PRP in the tube, the content was poured on the plate, so that it could cover its whole extension and turn solid as a carpet, therefore named as Carpet gel.

The culture medium employed was the DMEM High Glicose (Gibco®) 10% bovine fetal serum (Gibco®) supplemented by L-glutha-min (Gibco®) and antibiotics/ antimycotics (Gibco®), which was refreshed once a week. The plates were kept in a 5% CO_2 heater (Thermo®) for the whole duration of the experiment. Photographs were taken (Canon PowerShotA620®) on the first three days and on the last day. The inspection of plates with a naked eye and under inverted microscope (Zeiss Axiovert®) was carried out on a daily basis. Furthermore, photographs were taken on the microscope in order to check the microstructure of the formed gels.

RESULTS

Platelets count ranged from 442 x 10^3/mm^3 to 513 x 10^3/mm^3. The time for gel formation ranged from 5 seconds (with 6X thrombin concentration) to 40-45 seconds (with the lowest concentrations – 1X and 2X). The gels lasted 20 days. The Sphere gel (Figure 1) showed opaque areas under the inverted microscope (Figure 2), while Carpet gel (Figures 3 and 4) did not. (Figure 5) The gel based on unfrozen platelets (Figure 6) has shown to be more translucent under microscope when compared to that based on fresh platelets.

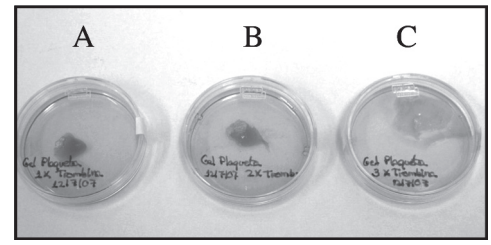

Figure 1 – *Thrombin1X, 2X and 3X Sphere gels, (A,B,C), respectively.*

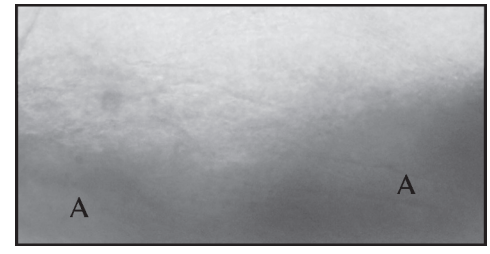

Figure 2 – *Microphotography of the Thrombin 1X Sphere gel. Note the opaque areas (A). Magnification 10x.*

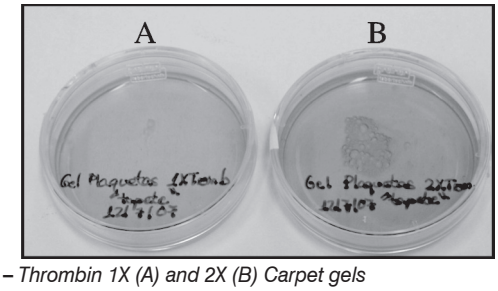

Figure 3 – *Thrombin 1X (A) and 2X (B) Carpet gels*

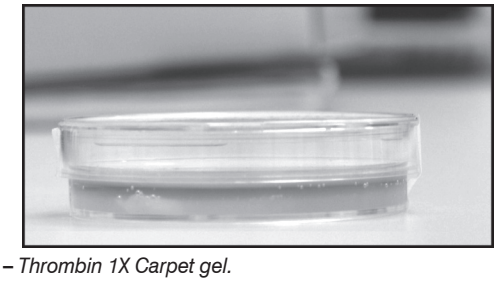

Figure 4 – *Thrombin 1X Carpet gel.*

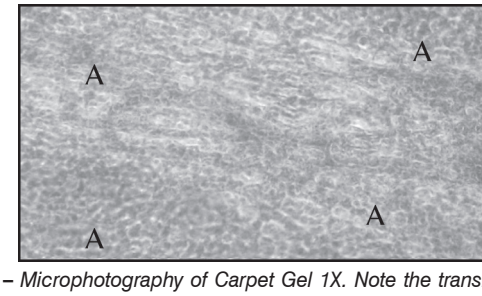

Figure 5 – *Microphotography of Carpet Gel 1X. Note the translucent areas (A). Magnification 10x.*

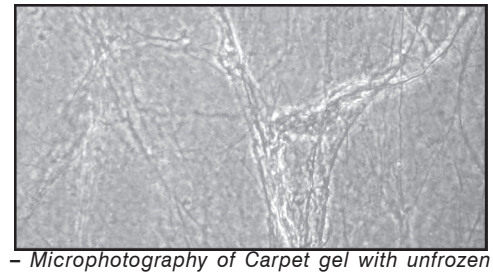

Figure 6 – *Microphotography of Carpet gel with unfrozen platelets. Magnification: 10x.*

DISCUSSION

All the produced gels achieved a solid state because of the action of calcium gluconate and thrombin. Both gels proposed here (Sphere and Carpet) showed a 3-D structure, so meeting the first requirement for a good scaffold. Both models evidenced positive and negative features: the Sphere gel did not adhere to the culture plate, thus it was easier to change the medium without damaging the gel, and, for conducting a histological analysis, we just had to remove it from the culture plate. However, visualizing it under the microscope is harder, due to the larger volume that produced the opaque areas seen on microphotographs (Figure 2), where any possibly existent cell was impossible to visualize. A reduction of gel size was found on the second day. In a pilot project to be conducted, PRP will be diluted before the Sphere gel is formed, in order to obtain a translucent gel. The Carpet gel has shown to be more homogenous, intact, and no reduction or opaque areas were seen under microscope. (Figure 5) In a cell culture process, this means a great advantage, since one of the tools is the daily analysis of plates by microscopy. As drawbacks, we can mention the adherence to the plate, challenging the replacement of a culture medium (especially with a vacuum pump); in addition, we can also mention the challenges of removing the gel from the plate at the moment of conducting histological analysis on it.

As for the thrombin concentrations used, we found that all of them were enough to activate platelet aggregation and form the gel. The only factor that varied upon the different concentrations was the gel forming time, which reduced when thrombin dosage was increased. This explains the use of only two concentrations tested on Carpet gels (1X and 2X).

The Carpet gel produced with double the PRP volume (4.6ml) was shown to be more rigid and easy to handle – compared to Carpet gels with 2.6 mL PRP – on culture medium changes for gel/ medium interface being more evident, without having impaired the daily investigation process by microscopy. The gel made with unfrozen platelets shows the advantage of providing better microscopic visualization conditions (translucent), due to platelets' rupture during unfreezing process, as well as the release of growth fractures of granules -α.

All gels remained stiff for 20 days in heater, when they were discharged. The twentieth day was regarded as the last day in this study, since three weeks are enough to conduct an experiment with cell culture, but gels can potentially have a longer useful life.

CONCLUSION

This study represents a technological innovation in the manufacturing of 3-D scaffolds for cell culture, because it pioneered the use of platelets directly on mechanical support to the cells. The advantages of platelets are evident in this science field compared to other several 3-D scaffolds currently used, many of which are already acclaimed, such as alginate pearls. To the authors, this first experience with platelets has shown to be quite promising, because we could successfully build two models of platelet gel use as 3-D scaffolds for cell culture: the Sphere and the Carpet. Both were shown to be effective to create 3-D environments, apparently porous, with some adjustments being required for improving the microenvironment for the cells in both models. The authors believe that the Sphere model can be improved only by using smaller PRP volumes (with the consequent formation of platelet pearls) or, either, with a dilution, what, in both cases, will lead to a reduced number of platelets employed and on a better visualization by inverted microscopy. However, only new experiments with the use of cells might answer this question. The Carpet gel may also be improved in the sense of seeking an optimal number of platelets providing good microscopic investigation conditions and a favorable microenvironment to the cells, being also warranted new experiments with cells to fulfill this objective. As final considerations, the present study opens a new path for science in terms of 3-D scaffolds production, which must be followed in order to be established as a new and efficient route in 3-D scaffold production technology for organ- and tissue-repairing medicine. The use of PRP on the scaffold seems to be a promising ally on cell culture, particularly cartilage cell culture, for two main reasons: 1) the possibility of using autologous material, where the scaffold is prepared with material removed from the patient him/herself, making the step of future implant integration easier; 2) for platelet and PRP being rich in cell growth factors that would act endogenously, not imposing other risks to patients.

We think that the platelet gel can be used as a 3-D scaffold in orthopaedics concomitantly to the autologous transplantation of chondrocytes, with the microencapsulation of chondrocytes previously expanded in cell culture, replacing currently employed scaffolds. Another future possibility would be the use of mesenchymal stem cells, differentiated as chondrocytes, using the scaffold described here, and then transplanting it to joint injury area.

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