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Research Article

Based Prosthetic Prototype of Polyester Implants for Labrum Reconstruction: Viability & Cell Attachment

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Abstract

Background: Synthetic grafts were developed to overcome problems related to autogenous grafts. The adhesive interactions of cells play a fundamental role in the healing process of ligament tissue engineering. One of the disadvantages of synthetic ligaments is the lack of biological cues for promoting cell adhesion and proliferation. The aim of this study is to evaluate the cell viability and adhesion to fibers of polyester implant for ligament tissue engineering and labrum reconstruction.

Methods: Mesenchymal stem cells were seeded ($10x10^5$) and cultured in an artificial prosthesis of polyester. Fragments were stained with calcein and photographs were taken at 24, 48, 72 and 120 hours, as well as later at two weeks in culture. The percentage of fluorescence was recorded using an Image J program.

Results: After two weeks of cell culture, the artificial prosthesis was covered by cells on average 98.57 ± 0.74% of the surface. The porous structure of the prosthesis was covered by a confluent layer of cells and extracellular matrix. Statistically significant differences were found between all the times analyzed (p=0.01).

Conclusion: Our results suggest that the cells seeded on the polyester prosthesis spread and proliferated until a confluent layer, showing that this has a good biocompatibility.

Keywords: Artificial prosthesis; Cell viability; Biocompatibility; Ligament reconstruction; Synthetic ligaments,

Introduction

It has been shown that the acetabular labrum has an important function in the normal biomechanics and stability of the joint. Labral tears are associated to a poor sealing of the joint fluid resulting in increased frictional forces and premature osteoarthritis [1,2]. The most common pathology in patients undergoing hip arthroscopy is labral tear [3]. When labral repair is not possible, debridement or reconstruction are indicated. Some authors have found that arthroscopic labral reconstruction is superior to labral resection in patients with irreparable or mostly calcified labrum with positive clinical outcomes [4]. Labrum reconstruction has recently become popular and multiple techniques using autografts or allografts reporting good outcomes at medium to long term

have been described [5,6]. However, both grafts types are not exempt from complications. Autografts have the disadvantage of donor site morbidity, inadequate graft sizing or morphology, otherwise allografts require procurement from a tissue bank, presents a potential risk of immune reaction and infectious diseases transmission, and have shown a later integration [7-11]. Only one study reported the use of synthetic graft for hip labrum reconstruction, this synthetic prosthesis is a polyurethane meniscal substitute adapted for augmentation and reconstruction of segmental labral tissue loss or irreparable labral damage [12]. Synthetic grafts were developed primary ligament reconstruction in the knee with the objective to overcome problems related to autologous and allogenic implants. Initially, some serious complications were reported with the use of those artificial prosthesis such as graft rupture, foreign-body, inflammation, and serious knee synovitis [13,14]. Although synovitis appears to be a rare complication, it is very serious and can result in ligament rupture and failure [15,16]. In the case of ligament reconstruction, an osteoarthritis case associated with LARS artificial ligament after anterior cruciate ligament surgery was reported [11]. In the histology of this report, the authors described only a few chondrocytes grew well along with the parallel fibers of the LARS ligament [11]. High graft failures, no so-called ligamentization and severe synovitis have been reported as major disadvantages of synthetic grafts [17-20]. The adhesive interactions of cells with other cells and the Extracellular Matrix (ECM) synthesis play a fundamental role in the healing process of ligament tissue engineering. At the cellular level, ligament wound healing involves cell attachment, detachment, migration, and proliferation. Cells and materials are two essential components in ligament tissue engineering, and so the interactions between them are important. Materials could interfere with cell adhesion, proliferation, and differentiation, while cell adhesion and subsequent functionality also affect properties of surrounding materials [21].

Pore interconnectivity throughout an implant favors the distribution of nutrients, cell migration, metabolic waste removal and the tissue ingrowth, enhancing its regenerative properties [22,23]. Contrasting to the natural materials, synthetic polymers present low immunogenicity potential and are more versatile. Polyesters has been effectively used to produce mechanically strong and biodegradable scaffolds for tendon/ligament applications [24,25]. These polymers are well characterized and have been approved by the FDA for certain human uses [26]. However, one of the disadvantages of synthetic polymers is the lack of biological cues for promoting cell adhesion and proliferation [26,27]. The aim of this study is to evaluate the cell viability and adhesion to fibers of polyester implant (PolyTape, Neoligaments™) for ligament tissue engineering.

Methods

Mesenchymal stem cells (ATCC-PCS-500-012, isolated from human bone marrow) were seeded and cultured in an artificial prosthesis of polyester at 37 °C in a 95% air and 5% CO_2 atmosphere**.** Before MSC seeding, the artificial prosthesis were sterilized in the flow hood with, then under sterile conditions in the mine flow hood, the polyester graft (PolyTape, Neoligaments™) was cut into 5mm fractions and placed in triplicate into wells of a 48-well plate. To initiate cell culture on the graft, $10x10^5$ cells were seeded in each fragment (5x7mm). First, each fragment was kept immersed in DMEM culture medium (Corning) during 10 minutes. Then, the culture medium was removed and with a micropipette, the cells suspended in 50 uL of culture medium were distributing them evenly over the entire surface of the scaffold. The synthetic prosthesis fragments were sterilized into the flow hood, then the cells were covered completely with DMEM-high glucose culture medium (Corning), supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic (Thermo Fisher Scientific). The changes of medium were carried out every two days for 2 weeks. To expose the proportion in which the cells colonized the surface of the prosthesis, the fragments were stained with calcein (caymanchem) at a concentration of 0.2 mg / mL. Cell growth was recorded with photographs at 24, 48, 72 and 120 hours, as well as later at two weeks in culture. The images were captured in a pyramid microscope Carl Zeiss Axio system image Vs 40X64 V. To obtain the percentage of fluorescence for calcein, 10 photos of each sample were taken, and Image J program (NIH) was used to obtain the mean of the fluorescence percentage for each sample.

Statistical Analysis

The data of this study were stored in an Excel data base (Microsoft Office for PC) and processed with the STATISTICA version 10 software. The percentages of fluorescence were obtained for the calcein at 24, 48, 72 and 120-hours. A Kolmogorov-Smirnov test was applied to establish if the samples presented a normal behavior and as a result of this the statistical significance of the differences between groups was determined by one-way Analyses of Variance (ANOVA); $p<0.05$ was statistically significant.

Results

Cell Growing at 2-Weeks

After two weeks of cell culture, the artificial prosthesis was evaluated by microscopy and was observed that cells covered the implant on average 98.57 ± 0.74% of the surface (Figure 1a).

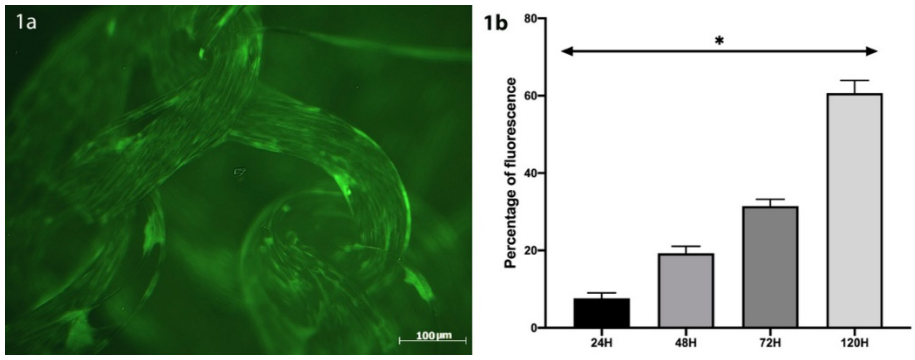

Figure 1: Photograph of calcein staining (green fluorescence) of mesenchymal stem cells cultured on the synthetic ligament fragment at two weeks of cell culture **(1a);** Graph of the analysis of the positive percentage to calcein staining at different times, where statistically significant differences were found between all the groups in hours that were analyzed ($p < 0.05$) **(1b).**

Cell Viability Quantified by Calcein Staining

Cell survival and proliferation was measured at 24, 48, 72, and 120 hours after seeding. The percentage of cell viability was assessed following 24 hours of incubation at 37 °C and 5% CO_2. Results showed a constant increase in cell density on the surface from day 1 until day 5. The porous structure was covered by a confluent layer of cells and extracellular matrix (Figure 2C). At 24 hours the 7.68 ± 1.12%, 48 hours the 19.26 ± 1.49%, 72 hours the 31.46 ± 1.43% and at the 120 hours the 60.65 ± 2.69%, on average were of percentages of green fluorescent quantified by calcein staining in the cells that were covering the superficial area of fragment plus cells (Figure 1b), we found statistically significant differences between all the times analyzed ($p=0.01$).

Cell Attachment to The Artificial Prosthesis

A well-defined architecture with same macropore structure of the artificial prosthesis was visualized by imaging of the green fluorescence. SEM observations allowed to determine that MSCs were able to adhere to the surface of the polyester scaffolds (Figure 2A & 2B). Cell morphology was fibroblast-like shape and those were spreading in the fibers surface. Further, it should be highlighted that there was no pore occlusion by the cells. However, by observing the pores it was shown that the cells were also capable of colonizing these areas, without occluding those.

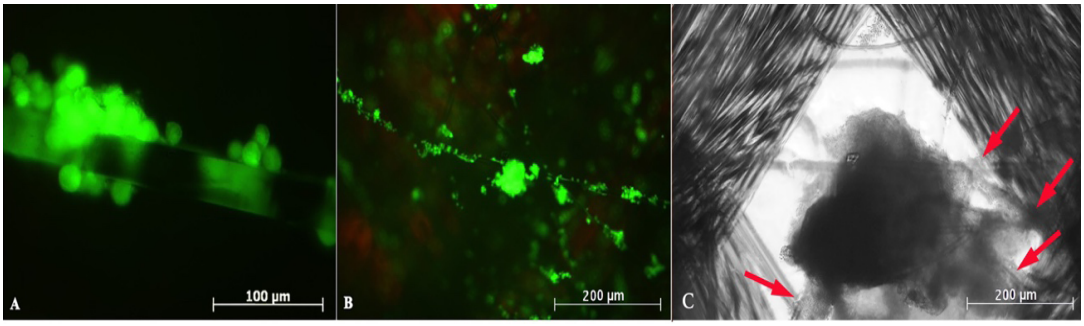

Figure 2: Morphology of polyester fibers and cells examined by fluorescent microscopy. **A:** Photography of polyester fibers. **B:** Images showing mesenchymal stem cells spread on the scaffold fibers. **C:** Visible light photomicrograph, where a cluster of growing cells is observed in a pore of the ligament scaffold, the arrows indicate the points of attachment.

Discussion

Cell adhesion into the structure and insertion site is an important factor in artificial ligament use. Various grafts have been used so far for the treatment of ligament reconstruction. The majority of synthetic grafts used in the past for knee have exhibited poor long-term physiologic and functional out-comes, no evidence in the literature is present about the use of those prosthesis for labrum reconstruction [28]. After trials in clinics for 20 years, most of these prostheses were no longer used because of high complication and failure rate (31%

to 42%) [29]. Generally speaking, early applications of artificial ligaments were not successful. The bleak results of follow-ups have revealed the underlying hazards: immune response, effusions, loosening, and rupture of the prostheses. However, recently novel types of artificial ligaments (Neoligaments) were also introduced in clinics, including artificial tendons and ligaments [30-34]. Neoligaments, prostheses made of polyester (Dacron), have been reported in clinical application with low rate failure [35]. Biocompatibility and mechanical strength are two key properties when we assess a scaffold used in a tissue-engineered ligament [36]. Tissue ingrowth is very important in artificial ligaments and is often affected by the surface topography, pore diameter, and porosity of the materials [37]. The device should also have interconnected porosity to allow cell migration, tissue growth, and vascularization within the tunnel segments.

In this study, an artificial flat ligament prepared with polyester fibers was used to investigate the effects of porous structures on cellular adhesion and migration. We observed that cells seeded on the polyester prosthesis spread and proliferated until a confluent layer forming extracellular matrix at two weeks of in-vitro culture on average 98.57% of the implant surface. Interestingly, we observed significant improvement in cell growing through the time reaching a 60.65% implant coverage at 5 days of culture quantified by percentages of green fluorescent by calcein staining. Those findings suggest a positive biocompatibility between mesenchymal stem cells showing that the polyester fibers are not cytotoxic. Cell accommodation through the scaffold suggests that its architecture, pores and surface provide a favorable environment for cell attachment. This also demonstrates that open architecture of the scaffold facilitates the infiltration of a cell suspension into the 3D structure of synthetic prosthesis. It was shown that interconnectivity of pores allowed for uniform cell distribution throughout the ligament, resulting in high cell density and homogeneous distribution at the end of the culture period. These new options could display the biology, integration and mechanics of the original labrum or ligament while sponsoring the growth of new tissue and resisting rejection from the body.

Conclusions

Results showed an increase in the number of viable cells in the surface of the scaffold fibers throughout the culture period, indicating an adequate compatibility to the cells cultured in the polyester prosthesis offering a new scaffold for labrum and ligament regeneration.

References

1. Ferguson SJ, Bryant JT, Ganz R, Ito K (2000) The influence of the acetabular labrum on hip joint cartilage consolidation: a proelastic finite element model. J Biomech 33: 953-960.
2. Ferguson SJ, Bryant JT, Ganz R, Ito K (2000) The acetabular labrum seal: a proelastic finite element model. Clin Biomech (Bristol, Avon) 15: 463.
3. Byrd JW, Jones KS (2001) Hip arthroscopy in athletes. Clin Sports Med 20: 749-776.
4. Domb BG, El Bitar YF, Stake CE, Trenga AP, Jackson TJ, et al. (2014) Arthroscopic labral reconstruction is superior to segmental resection for irreparable labral tears in the hip: A matched-pair controlled study with minimum 2-year follow-up. Am J Sports Med 42: 122-130.
5. Lynch TS, Minkara A, Aoki S, Bedi A, Bharam S, et al. (2020) Best practice guidelines for hip arthroscopy in femoroacetabular impingement: results of a Delphi process. J Am Acad Orthop Surg 28: 81-89.
6. Kyin C, Maldonado DR, Go CC (2020) Mid- to long-term out- comes of hip arthroscopy: a systematic review. Arthroscopy 2020.
7. White BJ, Herzog MM (2015) Labral reconstruction: when to perform and how. Front Surg 2: 27.
8. Atzmon R, Radparvar JR, Sharfman ZT, Dallich AA, Amar E, et al. (2018) Graft choices for acetabular labral reconstruction. J Hip Preserv Surg 5: 329-338.
9. Moya E, Natera LG, Cardenas C, Astarita E, Bellotti V, et al. (2016) Reconstruction of massive posterior nonrepairable acetabular labral tears with peroneus brevis tendon allograft: arthroscopy-assisted mini-open approach. Arthrosc Tech 5: e1015-22.
10. Domb BG, Gupta A, Stake CE, Hammarstedt JE, Redmond JM (2014) Arthroscopic labral reconstruction of the hip using local capsular autograft. Arthrosc Tech 3: e355-359.
11. Du Y, Dai H, Wang Z, Shi Ch, Xiao T, et al. (2020) A case report of traumatic osteoarthritis associated with LARS artificial ligament use in anterior cruciate ligament reconstruction. BCM Musculoskeletal Disorders 21: 2-6.
12. Marc Tey-Pons, Bruno Capurro, Raúl Torres-Eguia, Fernando Marqués-López, Alfonso Leon-García, et al. (2021) Labral reconstruction with polyurethane implant. Journal of Hip Preservation Surgery 8: 34-40.
13. Parchi PD, Ciapini G, Paglialunga C, Giuntoli M, Picece C, et al. (2018) Anterior cruciate ligament reconstruction with LARS artificial ligament- clinical results after a long-term follow-up. Joints 6: 75-79.
14. Ochen Y, Beks RB, Emmink BL, Wittich P, van der Velde D, et al. (2020) Surgical treatment of acute and chronic AC joint dislocations: Five-year experience with conventional and modified LARS fixation by a single surgeon. J Orthop 17: 73-77.
15. Doganavsargil B, Pehlivanoglu B, Bicer EK, Argin M, Bingul KB, et al. (2015) Black joint and synovia: Histopathological evaluation of degenerative joint disease due to Ochronosis. Pathol Res Pract 211: 470-477.
16. Bleedorn JA, Greuel EN, Manley PA, Schaefer SL, Markel MD, et al. (2011) Synovitis in dogs with stable stifle joints and incipient cranial cruciate ligament rupture: a cross-sectional study. Vet Surg 40: 531-543.
17. Ventura A, Terzaghi C, Legnani C, Borgo E, Albisetti W (2010) Synthetic grafts for anterior cruciate ligament rupture: 19-year outcome study. The Knee 17: 108-113.

18. Glezos CM, Waller A, Bourke HE, Salmon LJ, Pinczewski LA (2012) Disabling synovitis associated with LARS artificial ligament use in anterior cruciate ligament reconstruction: a case report. Am J Sports Med 40: 1167-1171.

19. Iliadis DP, Bourlos DN, Mastrokalos DS, Chronopoulos E, Babis GC (2016) LARS artificial ligament vs ABC purely polyester ligament for anterior cruciate ligament reconstruction. Orthop J Sports Med 4: 1-10.

20. Zhen-Yu Jia, Chen Zhang, Shi-qi Cao, Chen-chen Xue, Tian-ze Liu, et al. (2017) Comparison of artificial graft versus autograft in anterior cruciate ligament reconstruction: a meta-analysis. BMC Musculoskeletal Disorders 18: 309.

21. Massia SP, Hubbell JA (1990) Covalently attached GRGD on polymer surfaces promotes biospecific adhesion of mammalian cells. Ann NY Acad Sci 589: 261-270.

22. Cooper JA, Lu HH, Ko FK, Freeman JW, Laurencin CT (2005) Fiber-based tissue engineered scaffold for ligament replacement: design considerations and in vitro evaluation. Biomaterials 26: 1523-1532.

23. Kwansa AL, Empson YM, Ekwueme EC, Walters VI, Freeman JW, et al. (2010) Novel matrix based anterior cruciate ligament regeneration. Soft Matter 6: 5016-5025.

24. Goh JCH, Sahoo S (2010) Scaffolds for tendon and ligament tissue engineering. In: Archer C, Ralphs J, editors. Regenerative medicine and biomaterials for the repair of connective tissues. Cambridge: Woodhead Publishing 2010: 452-468.

25. Ge Z, Goh JC, Wang L, Tan EP, Lee EH (2005) Characterization of knitted polymeric scaffolds for potential use in ligament tissue engineering. J Biomater Sci Polym 16: 1179-1192.

26. Beldjilali-Labro M, Garcia Garcia A, Farhat F, Bedoui F, Grosset JF, et al. (2018) Biomaterials in tendon and skeletal muscle tissue engineering: current trends and challenges. Materials 11: 1116.

27. Janoušková O (2018) Synthetic Polymer Scaffolds for Soft Tissue Engineering. Physiol Res 67: S335-S348.

28. Mowbray M (2001) Reconstruction of the anterior cruciate ligament of the knee using an artificial ligament. Surg Tech Orthop Traumatol B 10: 55-540.

29. Rushton N, Dandy DJ, Naylor CP (1983) The clinical, arthroscopic and histological findings after replacement of the anterior cruciate ligament with carbon-fiber. J Bone Joint Surg Br 65: 308-309.

30. Paulos LE, Rosenberg TD, Grewe SR, Tearse DS, Beck CL (1992) The GORE-TEX anterior cruciate ligament prosthesis: A long-term follow-up. Am J Sports Med 20: 246-252.

31. Woods GA, Indelicato PA, Prevot TJ (1991) The Gore-Tex anterior cruciate ligament prosthesis. Two versus three-year results. Am J Sports Med 19: 48-55.

32. Muren O, Dahlstedt L, Dalén N (2003) Reconstruction of acute anterior cruciate ligament injuries: a prospective, randomized study of 40 patients with 7-year follow-up. No advantage of synthetic augmentation compared to a traditional patellar tendon graft. Arch Orthop Trauma Surg 123: 144-147.

33. Maletius W, Gillquist J (1997) Long-term results of anterior cruciate ligament reconstruction with a Dacron prosthesis. The frequency of osteoarthritis after seven to eleven years. Am J Sports Med 25: 288-293.

34. Nada AN, Debnath UK, Robinson DA, Jordan C (2010) Treatment of massive rotator-cuff tears with a polyester ligament (Dacron) augmentation: clinical outcome. J Bone Joint Surg Br 92: 1397-1402.

35. Xu HH, Simon CG Jr (2005) Fast setting calcium phosphate-chitosan scaffold: mechanical properties and biocompatibility. Biomaterials 26: 1337-1348.

36. Petrie RJ, Doyle AD, Yamada KM (2009) Random versus directionally persistent cell migration. Nature Reviews Molecular Cell Biology 10: 538-549.

37. Estevez M, Martinez E, Yarwood SJ, Dalby MJ, Samitier J (2015) Adhesion and migration of cells responding to microtopography. Journal of Biomedical Materials Research Part A 103: 1659-1668.