The Effect of Three Different Suture Anchors for Rotator Cuff Repair on Primary Cultures of Human Bone Marrow Mesenchymal Stem Cells

Gabriele Thiébat1 Paolo Capitani1 Laura de Girolamo2 Carlotta Perucca Orfei2 Francesca Facchini1 Herbert Schoenhuber1 Marco Viganò2

1Sport Traumatology Centre, Istituto Ortopedico Galeazzi, Milano, Italy
2Orthopaedic Biotechnology Laboratory, Istituto Ortopedico Galeazzi, Milano, Italy

Joints 2018;6:100–103.

Abstract

Purpose The purpose of this study is to investigate the in vitro biocompatibility of three different suture anchors (all-suture anchor, metal anchor, and polyetheretherketone anchor), commonly used for the rotator cuff repair.

Methods To assess the biocompatibility of the anchors, the possible cytotoxicity and the immunogenicity of the devices were assessed by cell viability assay and cell count on cultures of bone marrow stem cells (BMSCs) and peripheral blood leucocytes (PBLs), respectively. The possible inhibitory effect of the devices on BMSCs osteogenic potential was evaluated by alkaline phosphatase activity and matrix deposition assay.

Results The viability of BMSCs was slightly reduced when cultured in the presence of the devices (−24 ± 3%). Nevertheless, they were able to differentiate toward the osteogenic lineage in all culture conditions. The proliferation of PBLs and the production of interleukin-2 were not enhanced by the presence of any device.

Conclusion The analyzed devices did not significantly affect the normal cells functions when directly cultured with human primary BMSCs or PBLs, in terms of osteogenic differentiation and inflammatory reaction.

Clinical Relevance A deeper knowledge of the biological reactions to different devices used in rotator cuff surgeries would improve the clinical outcome of these procedures.
In vitro Biocompatibility of Anchors for Rotator Cuff Surgery

Thiébat et al.

this type of suture anchors, such as suture failure due to early degradation, reactive synovitis, cyst formation, and osteolysis. Moreover, standard radiographs do not allow for the visualization of a mobilized biodegradable anchor, and MRI would be needed.

The development of these devices continued to move forward and new anchors were recently introduced by using new materials. Nevertheless, little consideration has been given to the possible interaction between these fixation devices and the biological environment. Indeed, a low biocompatibility could represent a trigger for inflammation or bone resorption, possibly causing failure of the procedure.

The purpose of this study was to verify the in vitro biocompatibility of three different types (all-suture anchor, metal anchor, and polyetheretherketone [PEEK] anchor). The hypothesis of the study was that suture anchors can differ according to the material in terms of cytotoxicity, immunogenicity, and inhibitory action on osteogenic differentiation.

Methods

Bone Marrow Stem Cells Isolation and Culture

Human bone marrow stem cells (BMSCs) were collected from waste surgical samples at our institute under written consent of the patients (M-SPER-014.ver7 for the use of surgical waste). Cells were obtained from the femoral canal of one donor after hip surgical replacement. The aspirate was washed with phosphate-buffered saline (PBS) and centrifuged at 623 g for 10 minutes. The pellet containing the mononuclear cells fraction was resuspended in complete medium (CM), composed of Dulbecco’s Modified Eagle Medium (DMEM) High Glucose (Sigma-Aldrich, St. Louis, Missouri, United States), 10% fetal bovine serum (FBS, Sigma-Aldrich), 50 U/mL penicillin, 50 mg/mL streptomycin, 2 mM L-glutamine (Life Technologies, Carlsbad, California, United States) and plated in culture flasks at a density of $5 \times 10^3$ cells/cm$^2$. Cells were maintained in culture and expanded until passage 4.

BMSCs were incubated with three different types of suture anchors: an all-suture anchor (JuggerKnot; Biomet, Warsaw, Indiana, United States), a metal anchor (ThRevo-FT; ConMed), and a PEEK anchor (Cross-FT; ConMed). BMSC cultures without the devices, was assessed after 7 days of culture by MTT assay. If signiﬁcantly lower viability was observed for BMSCs incubated with the devices ($p < 0.05$), the hypothesis was that suture anchors can differ according to the material in terms of cytotoxicity, immunogenicity, and inhibitory action on osteogenic differentiation.

Osteogenic Differentiation

At passage 4, BMSCs were seeded at the density of $10^4$ cells/cm$^2$ in a 12-well plate. Osteogenic stimulus was provided by culturing cells in CM supplemented with dexamethasone (10 nM), glycerol 2-phosphate (10 mM), L-ascorbic acid 2-phosphate (150 µM) and cholecalciferol (10 nM). Cultures with each device were performed in duplicates and BMSCs without devices were considered as controls. Alkaline phosphatase (ALP) activity was assessed after 14 days of culture, by enzymatic assay. Briefly, specimens were lysed in 0.1% Triton X-100 and incubated at 37°C with 1 mM para-nitrophenyl phosphate in phosphate buffer (100 mM diethanolamine and 0.5 mM MgCl$_2$). The amount of chromogenic para-nitrophenol (pNP) product was assessed by spectrophotometer. Readings were taken at 405 nm wavelength, and ALP activity was normalized against the total protein content determined by bichoninic acid (BCA) protein assay (BCA Protein Assay Kit; Thermo Fisher Scientific, Waltham, Massachusetts, United States).

Calciﬁed matrix deposition was assessed after 21 days of culture, by alizarin red staining (ARS). Cells were stained with 40 mM Alizarin Red S for 15 minutes. Calcified matrix-bound dye was extracted by incubation with cetylpyridinium chloride (CPC) in phosphate buffer (0.1 M) to quantify the amount of calcified matrix. Spectrophotometer readings were taken at 550 nm wavelength.

Peripheral Blood Leucocytes Isolation and Culture

Human peripheral blood leucocytes (PBLs) were isolated from 10 mL of peripheral blood from one healthy donor, under informed consent. Briefly, blood was diluted 1:1 with PBS layered over 10 mL Ficoll Isopaque (GE Healthcare) in a 50-mL tube and centrifuged for 40 minutes at 400 g. After centrifugation, the nucleated cells at the interface between Ficoll and supernatant were collected, washed two times with PBS, counted, and then maintained in RPMI medium with 10% FBS. Cells were then cultured in the presence or absence of the three different devices for 5 days.

Data Analysis

Data from cell count, U/µg of ALP activity, absorbance at 570 and 550 nm for cell viability and ARS staining, respectively, were expressed as means ± standard deviation. Statistical analyses were performed with a statistical software (GraphPad Prism v5.00; GraphPad Software, La Jolla, California, United States) using the analysis of variance one-way test with Bonferroni’s post hoc test for the evaluation of differences between single datasets. Level of signiﬁcance was set at $p < 0.05$.

Results

Cell Viability

Cell viability of BMSCs, incubated or not with the medical devices, was assessed after 7 days of culture by MTT assay. If compared with the control samples (without devices), significantly lower viability was observed for BMSCs incubated with ThRevo-FT ($-22%$; $p < 0.05$) and Cross-FT ($-28%$; $p < 0.05$). However, no differences were observed among cultures incubated with the devices ($p > 0.05$).
Osteogenic Differentiation
The ALP activity levels of osteo-differentiated BMSCs, both in the presence or absence of the devices, were higher than the undifferentiated ones. In particular, significantly higher mean values were observed for BMSCs alone (**p < 0.05) and for BMSCs incubated with the ThRevo-FT anchors (±40%; p < 0.05). A nonsignificant increase of ALP activity was observed in samples in contact with JuggerKnot with respect to the control maintained in noninductive medium (p = 0.069). However, osteo-differentiated BMSCs cultured in the presence of the three different devices showed lower levels of ALP activity in comparison with osteo-differentiated BMSCs alone (Fig. 2).

As expected, the deposition of calcified matrix of differentiated BMSCs was higher than one of the undifferentiated cells (+1551%; p < 0.001). Similarly, BMSCs cultured with the devices produced higher levels of calcified matrix in comparison with control samples, even if only tendency were found in the statistical analysis, due to high variability in the samples. In particular, increases of 1.238% (p = 0.057), 1.277% (p = 0.096), and +1.320% (p = 0.096) were observed for JuggerKnot, ThRevo-FT, and Cross-FT, respectively (Fig. 3).

Immune Response Activation
After 5 days of culture, cells were quantified by the Trypan blue staining method. The PBLs counts in the samples incubated with the devices were similar to those from the control group; hence, PBLs proliferation was not increased by the presence of the devices. On the contrary, slight decreases in the number of PBLs were detected in all the samples cultured in the presence of the devices (Fig. 4). Interleukin-2 content was determined by enzyme-linked immunosorbent assay after 5 days in culture and it resulted undetectable for all specimens.

Discussion
Rotator cuff repair aims to restore the bone–tendon contact interface. Open and the arthroscopic transosseous repair technique restore the bone–tendon interface without the use of implant devices, other than the suture thread. However, standard arthroscopic rotator cuff repair took
advantage of implantable devices, the suture anchors. These
devices are within the bone–tendon interface and they
should not hinder tendon-to-bone healing process. Osteo-
lysis and cysts, described among the complications in the use
of these devices, were supposed to have not only mechanical
causes but also local biological causes in the interaction of
the anchors with bone and soft tissues.\(^7\),\(^14\)

Some previous studies focused on the different effects
of these devices on the surrounding cellular and tissue environ-
ment. Unfortunately, several variables are involved in the
modulation of healing processes and cellular signals, related
to the implants and to the patients as well. Zhang et al\(^15\)
reported that age and sex affect the ability of osteoblasts to
produce bone. Furthermore, injured tendons were found to
have different collagen-type composition compared with non-
injured ones.\(^16\)

Numerous modifications of the devices concerning mate-
rial composition and coating have been proposed to improve
their osseointegration and tissue healing. A different bone
response, both in formation and in cells modulation, was found
to the implants and to the patients as well. Zhang et al
reported that age and sex affect the ability of osteoblasts to
produce bone. Furthermore, injured tendons were found to
have different collagen-type composition compared with non-
injured ones.\(^16\)

Mazzocca et al\(^18\) reported that a collagen-coated suture
stimulates primary human osteoblasts and tenocytes to
proliferate, to adhere, and to differentiate, compared with a
noncoated suture. This was the first study that focused on
the biocompatibility of suture anchors made of different
materials on the same cellular environment in vitro.

In this study, BMSCs cultured in the presence of all-suture,
metal, and PEEK anchors maintained their viability. Although
cell viability resulted slightly decreased in all the samples
cultured in the presence of the devices, if compared with the
control, such reduction may have been provoked by the
culture method. In fact, the direct contact of the devices
with the cells may have hampered the normal cell growth.

The presence of the devices in direct culture with BMSCs did
not prevent their differentiation ability toward the osteogenic
lineage. Indeed, when maintained in osteogenic inductive
medium, all the samples were able to deposit higher amount
of calcified matrix with respect to the undifferentiated control.
Similarly, ALP activity resulted higher in all samples, compared
with control cells in noninductive medium. Despite a compari-
son with control cells in osteogenic medium showed a reduc-
tion in these parameters, the entity of such a decrease is
insubstantial because all the samples resulted positive to
calcified matrix deposition and ALP activity increased with
respect to undifferentiated controls.

None of the investigated devices was found to elicit an
inflammatory response when incubated with PBLs. The
absence of PBL activation was confirmed by the lack of cell
proliferation and IL-2 production in all the analyzed samples,
supporting the nonimmunogenicity of the devices.

In conclusion, no relevant interference with normal cell
functions was detected when BMSCs or PBLs were cultured in
direct contact with all-suture, metal, and PEEK suture anchors.
Further in vitro studies are needed to better evaluate the local
effect of suture anchors and the biological pathways to reduce
complications and improve tendon healing.

Conflict of Interest
Dr. de Girolamo reports personal fees from Lipogems and
Geistlich and grants from IGEA, outside the submitted
work.

References
\(^1\) DeHaan AM, Axelrad TW, Kaye E, Silvestri L, Puskas B, Foster TE.
Does double-row rotator cuff repair improve functional outcome
of patients compared with single-row technique? A systematic
review. Am J Sports Med 2012;40(05):1176–1185
\(^2\) Randelli P, Randelli F, Compagnoni R, et al. Revision reverse
shoulder arthroplasty in failed shoulder arthroplasties for rotator
cuff deficiency. Joints 2015;3(01):31–37
\(^3\) Saccamanno MF, Siriana G, Cazzato G, Donati F, Randelli P, Milano
G. Prognostic factors influencing the outcome of rotator cuff
repair: a systematic review. Knee Surg Sports Traumatol Arthros
c 2016;24(12):3809–3819
\(^4\) Nagra NSN, Zarag N, Smith RDJ, Carr AJ. Mechanical properties
of all-suture anchors for rotator cuff repair. Bone Joint Res 2017;6
(02):82–89
\(^5\) Esquivel AO, Duncan DD, Dobrascvic N, Marsh SM, Lemos SE. Load
to failure and stiffness: anchor placement and suture pattern
effects on load to failure in rotator cuff repairs. Orthop J Sports
Med 2015;3(04):2325967115579052
\(^6\) Goeminne S, Debeer P. Delayed migration of a metal suture anchor
to the glenohumeral joint. Acta Orthop Belg 2010;76(06):834–837
\(^7\) Dhawan A, Ghodadra N, Karas V, Salata MJ, Cole BJ. Complications
of bioabsorbable suture anchors in the shoulder. Am J Sports
Med 2012;40(06):1424–1430
\(^8\) Kelly JD II. Disintegration of an absorbable rotator cuff anchor six
weeks after implantation. Arthroscopy 2005;21(04):495–497
\(^9\) Park AY, Hatch JD. Proximal humerus osteolysis after revision
rotator cuff repair with bioabsorbable suture anchors. Am J
Orthop 2011;40(03):139–141
\(^10\) Kim SH, Oh JH, Lee OS, Lee HR, Hargens AR. Postoperative imaging
of bioabsorbable anchors in rotator cuff repair. Am J Sports Med
2014;42(03):552–557
\(^11\) McCarty LP III, Buss DD, Datta MW, Freehill MQ, Giveans MR.
Complications observed following labral or rotator cuff repair
with use of poly-L-lactic acid implants. J Bone Joint Surg Am 2013;
95(06):507–511
\(^12\) Medina G, Garofo G, D’Elia CO, Bitar AC, Castropil W, Schor B.
Bioabsorbable suture anchor migration to the acromioclavicular joint:
how far can these implants go? Case Rep Orthop 2014;2014:834896
\(^13\) Randelli P, Stoppani CA, Zioin C, Menon A, Randelli F, Cabitza P.
Advantages of arthroscopic rotator cuff repair with a transosseous
duette technique: a prospective randomized controlled trial. Am J
Sports Med 2017;45(09):2000–2009
\(^14\) Luyckx T, Debeer P. Management of full thickness rotator cuff tears.
A survey amongst members of the Flemish Elbow and Shoulder
Surgeons Society (FLEESSS). Acta Orthop Belg 2010;76(01):14–21
\(^15\) Zhang H, Lewis CG, Arnow MS, Gronowicz GA. The effects
of patient age on human osteoblasts’ response to Ti-6Al-4V implants
in vitro. J Orthop Res 2004;22(01):30–38
\(^16\) Maffulli N, Ewen SW, Waterston SW, Reaper J, Barrass V. Teno-
cytes from ruptured and tendinopathic Achilles tendons produce
greater quantities of type III collagen than tenocytes from normal
achilles tendons. An in vitro model of human tendon healing. Am J
Sports Med 2000;28(04):499–505
\(^17\) Zeriqat H, Akin FA, Howlett CR, et al. Differentiation of human
bone-derived cells grown on GRGDSP-peptide bound titanium
surfaces. J Biomed Mater Res A 2003;64(01):105–113
\(^18\) Mazzocca AD, McCarthy MB, Arciero C, et al. Tendon and bone
responses to a collagen-coated suture material. J Shoulder Elbow
Surg 2007;16(5, Suppl):S222–S230