Properties of Biologic Scaffolds and Their Response to Mesenchymal Stem Cells

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Purpose: The purpose of this study was to examine, in vitro, the cellular response of human mesenchymal stem cells (MSCs) to sample types of commercially available scaffolds in comparison with control, native tendon tissue (fresh-frozen rotator cuff tendon allograft). **Methods:** MSCs were defined by (1) colony-forming potential; (2) ability to differentiate into tendon, cartilage, bone, and fat tissue; and (3) fluorescence-activated cell sorting analysis (CD73, CD90, CD45). Samples were taken from fresh-frozen human rotator cuff tendon (allograft), human highly cross-linked collagen membrane (Arthroflex; LifeNet Health, Virginia Beach, VA), porcine non–cross-linked collagen membrane (Mucograft; Geistlich Pharma, Lucerne, Switzerland), a human platelet-rich fibrin matrix (PRF-M), and a fibrin matrix based on platelet-rich plasma (ViscoGel; Arthrex, Naples, FL). Cells were counted for adhesion (24 hours), thymidine assay for cell proliferation (96 hours), and live/dead stain for viability (168 hours). Histologic analysis was performed after 21 days, and the unloaded scaffolds were scanned with electron microscopy. **Results:** MSCs were successfully differentiated into all cell lines. A significantly greater number of cells adhered to both the non–cross-linked porcine collagen scaffold and PRF-M. Cell activity (proliferation) was significantly higher in the non–cross-linked porcine collagen scaffold compared with PRF-M and fibrin matrix based on platelet-rich plasma. There were no significant differences found in the results of the live/dead assay. **Conclusions:** Significant differences in the response of human MSCs to biologic scaffolds existed. MSC adhesion, proliferation, and scaffold morphology evaluated by histologic analysis and electron microscopy varied throughout the evaluated types of scaffolds. Non–cross-linked porcine collagen scaffolds showed superior results for cell adhesion and proliferation, as well as on histologic evaluation. **Clinical Relevance:** This study enables the clinician and scientist to choose scaffold materials according to their specific interaction with MSCs.

The primary biomechanical strength of rotator cuff (RC) repair has been continually improved over recent years. However, healing of repaired RC tendon often results in the formation of fibrovascular scar tissue between the tendon and the bone.[1-3] This formation has been shown to diminish the mechanical properties of the repair, thereby making it prone to structural failure, which may negatively affect the clinical outcome.[1,4] The addition of human mesenchymal stem cells (MSCs) and growth factors to the repair zone may improve healing in RC repairs.[5-9]

MSCs have the potential to undergo self-renewal; can differentiate into bone, tendon, cartilage, and ligament (i.e., multipotency); and can prolong the release of growth factors.[6,10,11] These properties may be beneficial for re-establishing the physiologic tendon-to-bone interface. Current studies have shown methods for safe and rapid "onsite" harvesting and isolation of MSCs with a specific focus on RC repair.[12,13]

The application of MSCs usually requires integration of a biologic carrier (scaffold) into the repair construct to localize and maintain cells in the area of tendon-to-bone healing.[5,14] Such biologic carriers are defined by Derwin et al.[15] as temporary scaffolds intended to enhance and

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The authors report the following potential conflict of interest or source of funding: R.A.A. receives support from Arthrex, Biomet, and Soft Tissue Regeneration. A.D.M. receives support from a Donaghue Grant and Arthrex to support research and equipment and salary.

The University of Connecticut Health Center/New England Musculoskeletal Institute has received direct funding and material support for this study from Arthrex. The company had no influence on study design, data collection, interpretation of the results, or the writing of the final manuscript.

Presented at the 2013 Congress of the International Society of Arthroscopy, Knee Surgery & Orthopaedic Sports Medicine, Toronto, Canada, May 2013.

Received June 11, 2013; accepted November 19, 2013.

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0749-8063/13387/$36.00

http://dx.doi.org/10.1016/j.arthro.2013.11.020

accelerate the biology of tissue repair. To date, clinical research regarding the use of biologic scaffolds in RC repairs is limited.[16] Most of this research is primarily focused on the use of scaffolds as mechanical bridging constructs for irreparable tears of the RC.[17-19] However, recent biomechanical studies have shown the possibility of scaffold integration into the repair zone without jeopardizing the repair construct's biomechanical properties.[20] These studies have also highlighted the 2 principal methods of scaffold augmentation for RC repair: either as a biomechanical reinforcement of the repair site with the focus on improved load distribution or as a biologic augmentation with the aim of enhanced tissue healing. Research using scaffolds as biologic carriers for cell application is limited to few in vivo animal studies.[8,21,22] To evaluate the potential for scaffolds as cell carriers in RC repair, basic science research regarding the in vitro behavior of MSCs in combination with these scaffolds is needed to develop a basis for further controlled in vivo studies. Because various types of biologic scaffolds exist, it is important to evaluate the entire spectrum to allow the surgeon distinctive utilization according to their biologic capacities.

The purpose of this study was to examine, in vitro, the cellular response of human MSCs to sample types of commercially available scaffolds in comparison with control, native tendon tissue (fresh-frozen RC tendon allograft). Our hypothesis was that currently available biologic scaffolds would be suitable for cell application but would show significant differences in MSC adhesion, proliferation, and viability, as well as differences in their microstructure.

Methods

Experimental Rationale

The study was performed in close accordance to methods previous published by Shea et al.[23] Each of the scaffolds was examined by 5 experimental methods: (1) cell adhesion, (2) cell proliferation, (3) live/dead assay, (4) histologic analysis, and (5) scanning electron microscopy. The sample size was limited to 4 per assay.[23] To accomplish successful integration of the scaffold into the host tissue, the MSCs must attach to the scaffold, proliferate, produce matrix, and ultimately, migrate into the scaffold. The use of the different assays was intended to evaluate this broad spectrum of MSC reactions to the biologic scaffold. As a control, 2 samples of each scaffold that were not loaded with MSCs were incubated and treated according to the procedure for scaffolds loaded with MSCs.

Bone Marrow Aspiration and MSC Culture

Previous studies showed that, with regard to age and gender, bone marrow aspirated from the proximal humerus gave consistently uniform results in terms of the number of colony-forming units and prevalence of MSCs.[12,13,24] Therefore, to minimize variability according to various donors, bone marrow was aspirated from the proximal humerus during arthroscopic RC surgery from one 54-year-old male patient following previously published methods[12] (institutional review board No. 06 577 2).

After centrifugation, the top layer containing the nucleated cells and the MSCs was drawn up and brought to the laboratory for cellular counting.[12] Nucleated cells were plated on 100-mm^2 Primaria dishes (BD Laboratories, Franklin Lakes, NJ) at a concentration of 5×10^5 cells/9.6 cm^2 into control media containing phenol red free α-minimum essential medium (Invitrogen, Carlsbad, CA), 10% fetal bovine serum (Atlanta Biologicals, Atlanta, GA), and 0.1% penicillin/streptomycin (Invitrogen). After 7 to 10 days, non-adherent cells were removed, and colony-forming units were counted. MSCs were grown to confluence and expanded. Only second-passage cells were used for experimentation. According to current literature, the isolated MSCs were defined by their (1) colony-forming potential; (2) adhesion to tissue culture plastic; (3) ability to differentiate into tendon, cartilage, bone, and fat tissue; and (4) fluorescence-activated cell sorting (FACS) analysis for surface markers.[12,25-28]

FACS Analysis

FACS was used to ensure that cells obtained from the proximal humerus had surface markers characteristic of stem cells. Cells were trypsinized in 0.25% trypsin/EDTA at confluence, rinsed, and centrifuged. The pellet was resuspended in staining buffer containing 1% human serum, 1% bovine serum albumin, and 1% fetal bovine serum in phosphate-buffered saline solution. Cells were incubated with either phycoerythrin or fluorescein isothiocyanate antibodies, washed with staining buffer, and analyzed with a FACSCalibur system (BD Biosciences, San Jose, CA). To identify MSCs, phycoerythrin-conjugated mouse monoclonal anti-CD73 immunoglobulin G (IgG), anti-CD90 IgG, and fluorescein isothiocyanate–conjugated antimouse CD45 monoclonal IgG were obtained from BD Biosciences. These were chosen in correlation with the suggestions of the International Society for Cell Therapy.[25] All antibodies were reactive against human antigens. Testing with negative and positive controls confirmed the specificity of these antibodies.

Biologic Scaffolds

Scaffolds were chosen to represent the ends of the broad spectrum of commercially available products.[16,29] The biomechanical properties of specific scaffolds have been tested in a previous study to ensure that these materials can be integrated into the repair zone without jeopardizing the construct's biomechanical properties.[20]

Representatives of highly cross-linked human dermis scaffolds, non—cross-linked collagen scaffolds, and fibrin matrices were selected. Native tissue collected from a human RC tendon (fresh-frozen allograft) was used as a control. The fresh-frozen allograft tissue, which was used as a control, was obtained from a 41-year-old female tissue donor (Musculoskeletal Transplant Foundation, Edison, NJ). To represent a highly cross-linked human dermis allograft scaffold, the Arthroflex decellularized human dermis patch (LifeNet Health, Virginia Beach, VA) was used.[30] A bilayer collagen matrix (Mucograft; Geistlich Pharma, Lucerne, Switzerland) consisting of type I and III porcine collagen served as a representative of non—cross-linked collagen scaffolds.[31,32] As a representative of fibrin matrices, a combination of a platelet-rich plasma (PRP) product, a gelling agent (ViscoGel; Arthrex, Naples, FL), and a platelet-rich fibrin matrix (PRF-M) were used.[33] For both the PRP gel and the PRF-M, 40 mL of peripheral blood was harvested from one donor, a 24-year-old man (institutional review board No. 10-204-2). For the production of the PRP gel, 20 mL of peripheral blood was centrifuged at 1,500 rpm for 5 minutes and the platelet-rich suspension was drawn up. This suspension was then mixed at a ratio of 10:1 with thrombin and calcium chloride solution to create the PRP gel. For platelet-rich fibrin (PRF) production, 2 glass tubes were each filled with 10 mL of peripheral blood and centrifuged at 3,000 rpm for 10 minutes, which produced the PRF-M.[33] According to the classification of Dohan Ehrenfest et al.,[34] the PRP gel is a representative of the "pure platelet-rich fibrin" group because of the absence of leukocytes and high content of platelets. Because the PRF-M contents include a high number of platelets as well as leukocytes, it is classified as leukocyte- and platelet-rich fibrin.[35]

Cell Adhesion Assay

Four 5 × 5—mm samples of each of the 7 scaffold devices were placed into tissue culture plates (BD Biosciences), and MSCs were pipetted onto each sample at a concentration of 450,000 cells/0.1 mL. Cells were allowed to adhere to each scaffold for 30 minutes before the addition of media to the well. Samples were incubated for 24 hours and removed from the well. The adherent cells were removed from the samples by incubating with 0.5 mL of trypsin for 20 minutes at 37°C. The cells from each sample were counted 3 times each in a Coulter counter (Coulter Electronics, Hialeah, FL).[23]

Cell Proliferation Assay

After culturing of the scaffold samples for 24 hours, proliferating cells were labeled with 5 Ci/mL of tritium-labeled ([3H]) thymidine (NEN, Boston, MA), which incorporates and binds to nuclear DNA. At 48 hours, the samples were removed and washed twice for 5 minutes with 10% trichloroacetic acid to remove insoluble [3H]-thymidine. Cells were lysed for 10 minutes in 0.5N sodium hydroxide. The radioactive thymidine was incorporated into the DNA of dividing cells, and therefore an increase in radioactivity above the negative control directly correlates to cellular proliferation.[36] Radioactivity was measured in the lysates with a liquid scintillation counter (Packard Instrument, Downers Grove, IL), and this was repeated 3 times for consistency.[23,36]

Live/Dead Assay

Each sample was stained with 2-μmol/L calcein-AM and 4-μmol/L ethidium homodimer-1 in sterile phosphate-buffered saline solution for 30 minutes after 7 days of cell culture (Molecular Probes [Invitrogen], Eugene, OR). Samples were mounted in staining solution in glass-bottom micro-well dishes (MatTek, Ashland, MA) and examined for cell viability and cytotoxicity with a confocal microscope (LSM 510; Carl Zeiss, Jena, Germany). Viable cells were stained green, whereas dead cells were stained red.

Histologic Analysis

Four 5 × 5—mm samples of each scaffold were incubated for 21 days. Increasing concentrations of ethanol were used to dehydrate the specimens, and after clearing them in xylene, they were embedded in paraffin. Five-micrometer-thick cross sections of each sample were obtained and stained with hematoxylin-eosin. An Optiphot Nikon microscope (Nikon, Melville, NY) at a magnification of 10× and a handheld counter were used to determine the number of MSCs that had migrated into the scaffold. All samples were evaluated in an unbiased, blinded manner by 3 independent examiners. A standardized box (0.5 mm^2) was created for each slide of each biomaterial, and cells were counted within that box.

Scanning Electron Microscopy

Samples of each scaffold were sputter coated with gold/palladium for 20 seconds with a Polaron E5100 SEM Coating Unit (Quorum Technologies, Laughton, UK). Images were obtained with a JEOL JSM-6335F field emission scanning electron microscope (JEOL, Peabody, MA), by use of an accelerating voltage of 10 to 15 kV, at various magnifications.

Statistical Analysis

Means and standard deviations of the 4 measures per group and assay were compared within each experiment. One-way analysis of variance was used to compare group means for experiments, followed by Tukey post hoc tests for experiments with a statistically significant difference in means. $P \leq .05$ was used to determine statistical significance. All statistical analyses were performed with SPSS software (IBM, Armonk, NY).

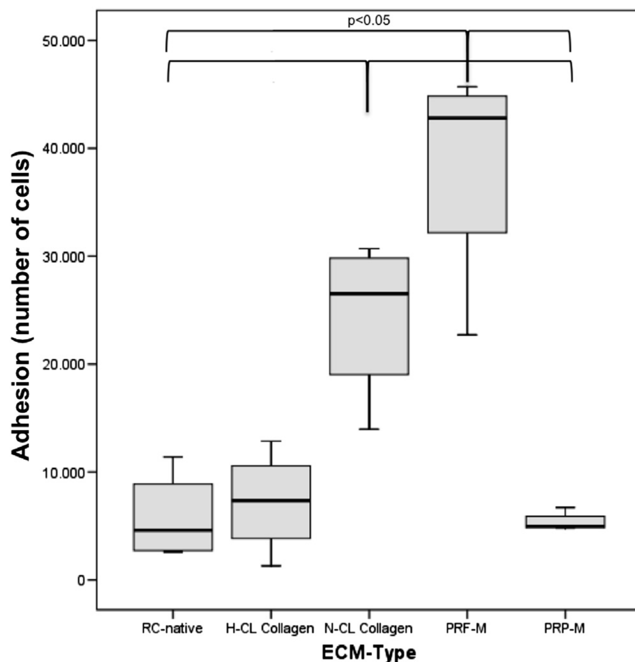

Fig 1. Results of adhesion assay showing significantly more cell adhesion for non−cross-linked (N-CL) collagen matrix and PRF-M. (ECM, extracellular matrix; H-CL, highly cross-linked; PRP-M, fibrin matrix based on PRP.)

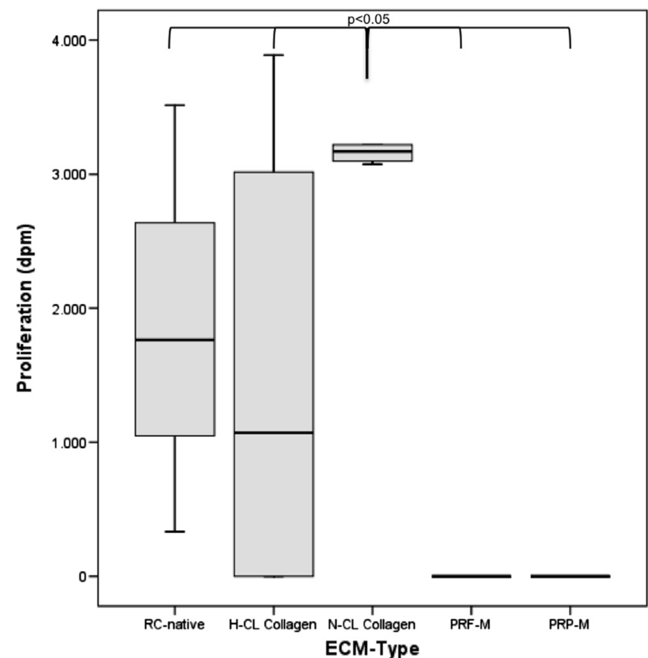

Fig 2. Results of proliferation assay showing significantly more proliferation (disintegrations per minute) for non−cross-linked (N-CL) collagen. (ECM, extracellular matrix; H-CL, highly cross-linked; PRP-M, fibrin matrix based on PRP.)

Results

MSC Culture

Before their use in this study, MSCs were successfully differentiated into cartilage, bone, tendon, and fat cells. The results of FACS analysis showed a high percentage of CD73 (93.2% ± 2.3%) and CD90 (88.4% ± 4.2%) surface markers and a minimal amount of CD45 markers (0.23% ± 1.2%), used as the negative control.

Cell Adhesion Assay

Figure 1 and Table 1 show the number of cells that adhered to the scaffold samples in 24 hours. A significantly greater number of cells adhered to the non−cross-linked collagen scaffold (24,425 ± 7,521 cells) and the PRF-M (38,500 ± 10,663 cells) compared with all other scaffolds and the native RC tendon (5,800 ± 4,107 cells) ($P \leq .05$). No significant difference was seen when the other scaffolds were compared (Table 1).

Cell Proliferation Assay

Figure 2 and Table 1 show the results of cell proliferation. Data are expressed as radioactive disintegrations per minute to describe the relative amounts of [3H]-thymidine incorporated into cellular DNA as a measure of cell division and proliferation. Cell proliferation was significantly higher in the non−cross-linked collagen scaffold compared with the PRF-M and the PRP matrix ($P \leq .05$). There were no significant differences when compared with the other scaffolds (Table 1).

Live/Dead Assay

The live/dead assay was performed after 7 days of cell incubation on the scaffold. Figure 3 shows

Table 1. Cell Adhesion and Proliferation Data According to Scaffold Type

	MSC Adhesion (Number of Cells)*				MSC Proliferation (Disintegrations per Minute)†			
			95% CI				95% CI	
	Mean	SD	Lower	Upper	Mean	SD	Lower	Upper
NC collagen	24,425	7,521	12,458	36,392	3,159	74	3,042	3,276
PRF-M	38,500	10,663	21,533	55,467	ND	ND	ND	ND
HC collagen	7,213	4,781	−3,956	14,821	1,507	1,881	−1,485	4,500
Native RC	5,800	4,107	−735	12,335	1,869	1,594	−2,089	5,828
PRP-M	5,375	892	3,955	6,795	ND	ND	ND	ND

CI, confidence interval; HC, highly cross-linked; NC, non−cross-linked; ND, not detectable; PRP-M, fibrin matrix based on platelet-rich plasma.
*Analysis of variance showed a statistically significant difference for adhesion: $P < .001$.
†Analysis of variance showed a statistically significant difference for proliferation: $P = .004$.

representative confocal microscopic images of the results of the live/dead assay. When we compared the cell viability (live/dead) of the MSCs cultured on the evaluated scaffolds, no significant differences were found ($P >$ .05). Cell viability of 100% ± 0% was found on both fibrin matrices, as well as the non−cross-linked collagen scaffold. After 7 days, 72% ± 38% of cells on the highly cross-linked collagen scaffold and 77% ± 37% of cells on the native RC tendon were viable.

Histologic Analysis

MSCs were detected on the borders of all scaffolds (Fig 4). The structure of the non−cross-linked as well as the highly cross-linked collagen membranes allowed cells to migrate into the structure of the scaffold. The highest number of cells was found in the non−cross-linked collagen scaffold (51 ± 5.6 cells/0.5 mm^2), and this was significantly higher than in all other scaffolds ($P <$ .001). The mean number of MSCs counted in the RC allograft was 28.3 ± 3 cells/0.5 mm^2. This was significantly higher compared with the highly cross-linked collagen (14.3 ± 4.5 cells/0.5 mm^2, $P = .01$), the PRP matrix (11 ± 2.6 cells/0.5 mm^2, $P = .002$), and the PRF-M (9.7 ± 2.1 cells/0.5 mm^2, $P = .001$). No significant differences were found when we compared the 3 other scaffolds with each other ($P >$ .99) (Figure 5).

Electron Microscopy

The morphology of native human RC tissue was found, on average, to have a porosity range of 20% to 30% by use of a porosimeter. The non−cross-linked collagen scaffold had a more open porous structure, with porosity in the 60% to 70% range. The porosity of the highly cross-linked collagen scaffold and fibrin matrices was in the same range as the native RC tissue.

Discussion

We observed significant differences in the reaction of human MSCs to biologic scaffolds in comparison with native tendon tissue (fresh-frozen allograft), which was used as a control. MSC adhesion, proliferation, and scaffold morphology evaluated by histologic analysis and electron microscopy also varied throughout the evaluated types of scaffolds. These findings are in agreement

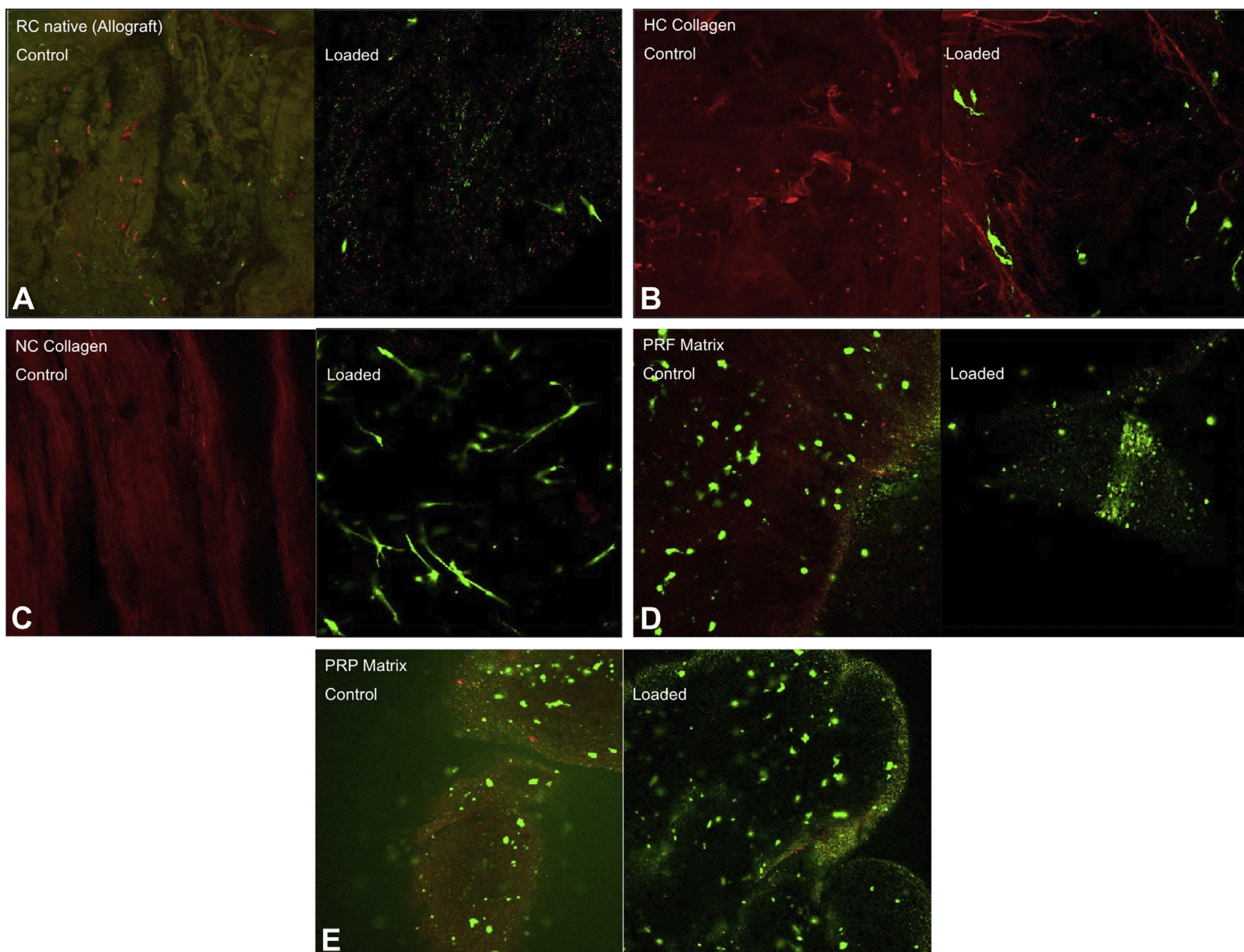

Fig 3. Confocal microscopic images of life/dead assay. (A) Native human RC tissue. (B) Highly cross-linked (HC) collagen scaffold. (C) Non−cross-linked (NC) collagen scaffolds. (D) PRF-M. (E) PRP matrix (PRP-M). (Magnification, ×10.)

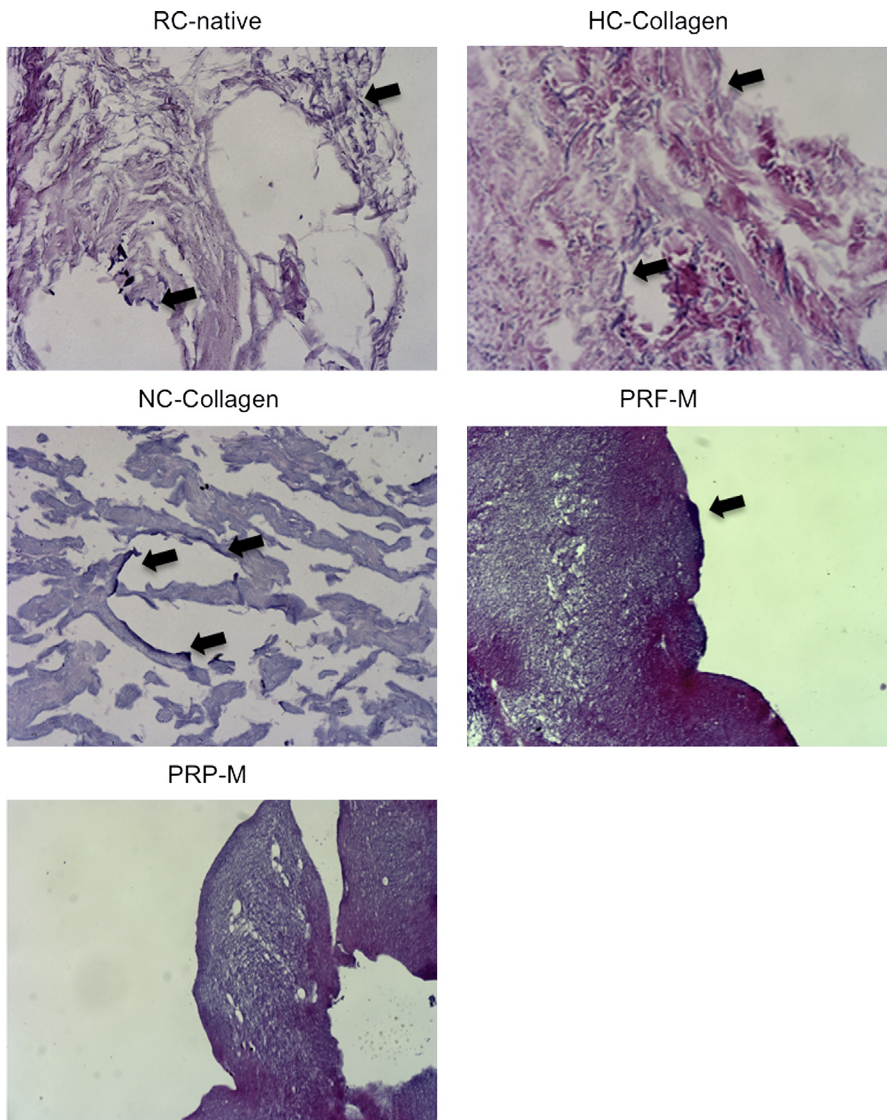

Fig 4. Representative hematoxylin-eosin–stained sections (magnification, ×4) of each scaffold sample. MSCs were detected on the borders of all scaffolds. (Arrows, MSCs; HC, highly cross-linked; NC, non–cross-linked; PRP-M, PRP matrix.)

with our hypothesis and suggest that obvious structural differences of scaffolds also result in differences in cellular properties and biological performance when loaded with MSCs.

There have been a number of previous reports on the in vivo data of each of the previously described scaffold groups.[5-7,9,17,19,21,37] However, most of these studies were performed in animal models, and clear data on the behavior of human MSCs on the various types of scaffolds are still lacking. Furthermore, the use of human MSCs permits detailed evaluation of species-specific reactions to the scaffold material. To allow for adequate and consistent examination of a variety of biologic scaffolds, bone marrow was harvested and isolated from only 1 single donor and a multistep laboratory testing protocol was performed to isolate MSCs. We believe that combining MSCs from more than 1 donor may confound the interaction between the cells and the MSCs. Testing for colony formation, tissue culture plastic adhesion, and multipotency and evaluation with FACS confirmed that the isolated cells were characteristic of MSCs.[12,25-28] Such methods for defining that cells are MSCs have been previously published, and it is generally agreed that they are indicative for this cell type.[14,25,28]

The evaluation of multiple assays (adhesion, proliferation, live/dead, histologic analysis, electron microscopy) was used to represent a wide spectrum of biologic properties of the evaluated scaffolds. Currently, no agreement exists on which assay is the most important indicator of successful integration of MSCs into the scaffold or for their application in in vivo studies. We used previously published methods and established techniques for our experimental setup to allow for comparability of our results across studies.[23] However, the determination of a favorable response by

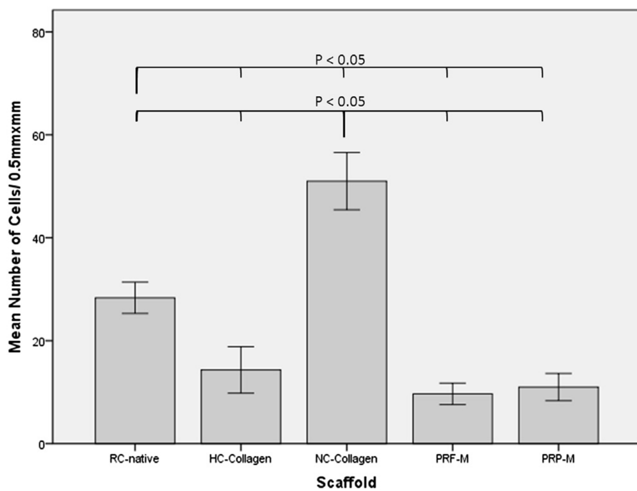

Fig 5. Results of cell count of scaffold samples showing mean number of cells/0.5 mm^2. (HC, highly cross-linked; NC, non−cross-linked; PRP-M, fibrin matrix based on PRP.)

the MSCs may be a summation of all experimental values. Finally, these "biological" properties have to be seen in correlation with the "biomechanical" properties known for these scaffolds if surgeons intend to integrate these scaffolds into the repair zone of an RC reconstruction.

Cell adhesion is based on a complex interaction between the scaffold microstructure and different types of cell surface receptors, namely integrins.[38] Establishment of functional transmembrane contacts with the scaffold's matrix is considered vital for MSC survival, proliferation, and eventual differentiation.[29] Integrins are a key component of MSC interaction with the scaffold, forming focal adhesion complexes that influence cytoskeletal dynamics and initiate various signaling cascades regulating MSC adhesion and migration on the scaffold.[39] This entire process is also dependent on the volume of cell suspension initially absorbed by the scaffold, which is regulated by the scaffold's microstructure (porosity).[16] We observed significantly greater amounts of cell adhesion with the non−cross-linked collagen scaffold (Mucograft). This may be because of the high content of type I collagen, which—along with fibronectin—facilitates MSC adhesion.[38] In addition, these scaffolds showed a porous structure with wide pore sizes on electron microscope evaluation. This characteristic permits for a rapid and more quantitative infusion of the cell suspension into the scaffold and therefore results in higher loads of initially loaded cells.[16] Such non−cross-linked scaffolds have been previously used as resorbable carriers in animal in vivo studies.[9,40]

We are aware of the current American Academy of Orthopaedic Surgeons guidelines on optimizing the management of RC repair.[41] The guidelines suggest that surgeons with a moderate recommendation not use a non−cross-linked porcine small intestine submucosal xenograft patch to treat patients with RC tears. This recommendation was based on the results of 2 studies performed by Iannotti et al.[42] (Level II) and Walton et al.[43] (Level III), who showed less favorable outcomes with the use of such grafts (Restore Orthobiologic Implant; DePuy Orthopaedics, Warsaw, IN) for the treatment of irreparable RC tears. They reported a hypersensitivity reaction rate of approximately 20% to 30% in their small groups of 10 and 16 patients.

The Restore Orthobiologic Implant consists, according to the manufacturer's information, of approximately 90% porcine collagen as well as porcine lipids and a small amount of carbohydrate. This is in contrast to the non−cross-linked scaffold used in our study (Mucograft), which consists of purified collagen obtained from pigs to avoid antigenic reactions. The clinical results of in vivo studies of the use of a very comparable scaffold (Chondro-Gide; Geistlich Pharma) for the treatment of cartilage defects in humans make us believe that immunologic reactions of the host might occur less than for the scaffolds containing animal lipids and carbohydrates. Such purified collagen scaffolds have been used for the treatment of cartilage defects in humans (autologous matrix−induced chondrogenesis) routinely and have shown promising results.[44,45] Further animal and human studies need to be performed to evaluate the individual immunologic reactions of these purified scaffolds. However, the significant limitation of the current basic science study is the inability to show immunologic reactions of the human host against the evaluated scaffolds, and this prohibits any conclusions on these questions.

The native RC tendon and the highly cross-linked collagen scaffold have a dense collagen structure with little matrix; this may be the reason for the less favorable results in terms of cell adhesion. Electron microscopy also showed the differences in porous structure of these scaffolds. Highly cross-linked collagen scaffolds produced from decellularized human dermis such as the Flexigraft (LifeNet Health), as well as other comparable patches, have been primarily used to mechanically augment RC repairs.[19,30,46] Snyder et al.[47] published histologic results of a biopsy specimen obtained 3 months after augmentation of an RC repair with an acellular dermis patch (GraftJacket Matrix-MaxForce Extreme; Wright Medical Technology, Arlington, TN). They were able to show that there was only little inflammatory response, but collagen fibers and blood vessel ingrowth showed that the graft exhibited a biological remodeling process. These scaffolds are produced from a densely structured decellularized human dermis, which results in increased biomechanical stability in comparison with non−cross-linked collagen scaffold.[37] The highly dense structure may have impeded MSC migration into the scaffold and possibly prevented MSC proliferation. Shea et al.[23] described similar observations for the migration of

tenocytes into scaffolds. Despite these findings, such scaffolds are used throughout the current in vivo studies mainly because of their biomechanical properties. Some authors have also reported on cell migration into the scaffold over time in certain in vivo experiments.[16,19,37] Surgeons have to be aware of the fact that such types of scaffolds allow for higher biomechanical stability but may have less ability to promote cellular migration compared with the non−cross-linked collagen scaffolds.

Fibrin matrices have been shown previously to be viable carriers for MSC application in tendon repair models.[7,48] The advantages of these scaffolds include their viscous structure and rapid resorption, as well as the additional growth factor content.[49] In contrast to the other scaffolds, fibrin-based matrices can be produced in an autologous onsite procedure from the patient's peripheral blood by an economically reasonable process. These scaffolds also have a high concentration of growth factors, as known from PRP products. Altogether, the previously mentioned factors make fibrin scaffolds interesting for the surgeon. However, the specific structure of these types of scaffolds makes them more attractive for the biological enhancement of the repair rather than biomechanical augmentation. Barber et al.[50] found lower retear rates on magnetic resonance imaging for RC repairs augmented with a PRF-M construct than without the augmentation. However, clinical scores failed to show significant differences except for the Rowe score. Interestingly, the fibrin scaffolds evaluated in our study allowed only nondetectable amounts of MSC proliferation. Although proliferation in the loaded scaffold was detected by the initial assay, no MSC proliferation was detectable when the results were corrected by subtracting the "background noise" of the unloaded scaffold. Bensaid et al.[51] reported that MSCs adhere, spread, and proliferate when loaded into fibrin scaffolds, provided that the concentration of fibrinogen was not higher than 18 mg/mL. If the fibrin concentration was higher, MSCs failed to further proliferate. Because of our setup, we were not able to evaluate the fibrin concentration of our scaffolds. However, the fibrin content of the scaffolds may have had such an effect on the MSCs. Together, the high amount of MSC adhesion and the known content of growth factors are seen as the main advantageous factors for the clinical use of such scaffolds, although the absence of biomechanical augmentation of the initial repair has to be expected.[49]

Limitations

This study has several limitations. The in vitro behavior of the evaluated cells may not mimic the in vivo environment of degenerated RC tears. This study was intended to give adhesion, proliferation, and histologic data on the utility of currently available scaffolds as carriers for MSCs. MSCs obtained from a single donor were isolated and cultured to allow for a very standardized evaluation of the different scaffolds. On the one hand, this allows for a very controlled experimental setup and comparable results, but on the other hand, donor-specific effects could not be ruled out entirely. The MSCs acquired from concentrated bone marrow after cell culture represented a heterogeneous cell population, and the determination of the 4 standardized characteristics of MSCs (colony formation, tissue culture plastic adhesion, differentiation potential, and FACS) distinguished the cell types.[12,25-28] This controlled that the cells obtained showed commonly accepted criteria for MSCs. Furthermore, this study was specifically planned to show the in vitro behavior of MSCs loaded on different available types of biologic scaffolds. Our purpose was to evaluate the ends of the spectrum of available scaffold types. We are aware of the numerous variations of biologic scaffolds that are commercially available. The intention of this study was to evaluate vastly different scaffolds that represent the spectrum of commercially available types. Including more samples would have been beyond the scope of our study. However, the data generated by this evaluation allow surgeons to better characterize these scaffolds and distinguish between the intended biological and biomechanical properties for integration into the RC repair. Evaluation of inflammatory responses of the host to the scaffold was not possible because of the study setup. This would be an interesting question to answer because previous authors have reported on immunologic reactions after the application of porcine scaffolds in the repaired RC. Overall, the potential of biological treatments using such combinations of scaffolds and MSCs requires further in vivo investigation. In this regard, the data resulting from our study may form the basis for future studies.

Conclusions

Significant differences in the response of human MSCs to biologic scaffolds existed. MSC adhesion, proliferation, and scaffold morphology evaluated by histologic analysis and electron microscopy varied throughout the evaluated types of scaffolds. Non−cross-linked porcine collagen scaffolds showed superior results for cell adhesion and proliferation, as well as on histologic evaluation.

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