Enhanced osteoblast-like cell adhesion and proliferation using sulfonate-bearing polymeric scaffolds

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Abstract: Orthopedic malfunction, degeneration, or damage remains a serious healthcare issue despite advances in medical technology. Proactive extracellular matrix (ECM)-mimetic scaffolds are being researched to orchestrate the activation of diverse osteogenic signaling cascades, facilitating osteointegration. We hypothesized that sulfonated functionalities incorporated into synthetic hydrogels would simulate anionic, sulfate-bearing proteoglycans, abundant in the ECM. Using this rationale, we successfully developed differentially sulfonated hydrogels, polymerizing a range of sulfopropyl acrylate potassium-acrylamide (SPAK-AM) mole ratios as monomer feeds under room temperature conditions. For anchorage-dependent cells, such as osteoblasts, adhesion is a critical prerequisite for subsequent osteointegration and cell specialization. The introduction of the sulfonated monomer, SPAK, resulted in favorable uptake of serum proteins with proportional increase in adhesion and proliferation rates of model cell lines, which included NIH/3T3 fibroblasts, MG-63 osteoblasts, and MC3T3-E1 subclone 4 preosteoblasts. In fact, higher proportions of sulfonate content (pSPAK75, pSPAK100) exhibited comparable or even higher degrees of adhesion and proliferation, relative to commercial grade tissue culture polystyrene *in vitro*. These results indicate promising potentials of sulfonated ECM-mimetic hydrogels as potential osteogenic tissue engineering scaffolds. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res 83A: 990–998, 2007

Key words: hydrogel; adhesion; proliferation; ECM; osteointegration

INTRODUCTION

Bone tissue malfunction, degeneration, and damage remain serious problems in healthcare despite advances in medical technology. In the United States, 2.5 million orthopedic and plastic reconstructions including bone, cartilage, tendon, ligament, and breast are performed annually.[1] Of bone fracture reconstructive surgeries, ~1 million surgeries have been identified to have a risk of fracture nonunion, delayed union, and osteomyelitis which typically result in substantial rates of complication and mortality.[2] Demographic factors underpin the development of the orthopedic implant market, with growth driven by an increasingly aging population suffering from afflictions requiring implants. Projections estimate that, as the baby boomer generation ages, there will be almost 11 million more people aged 60 or greater in 2010 than were in 1995.[3] Further, osteoporosis, the loss of bone and attendant increased risk of fracture, occurs with age, particularly in women.

The interdisciplinary area of tissue-engineering is being increasingly explored to overcome these complicating factors associated with bone replacement. With increased understanding of the causes of acceptance or rejection of an artificially engineered tissue, a paradigm shift has taken place in bone tissue reconstruction—from the use of inert, nontoxic scaffolds to the use of proactive scaffolds simulating the extracellular machinery of bone.[4–6] Biomimetic material chemistry has the potential to reproduce in synthetic systems aspects of the complicated natural machinery of living tissues. In fact, biomimetic strategies do not directly transcribe the properties of their natural counterparts; they aim to abstract key concepts from the biological tissues to be adapted in a synthetic context. The use of biomimetic scaffolds facilitates the acceptance of engineered systems by the body through a more natural cell–material interaction.[7–12] Acceptance by the body may include decreased immune system recognition[13] or stimulated

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cell attachment, adhesion, proliferation, and differentiation due to the microenvironment.[14]

Since the pioneering work of Wichterle and Lim on hydrogels for ophthalmic applications,[15] biomaterial scientists have actively researched polymeric hydrogels for biomedical applications, including recent research in tissue engineering. One major reason for utilizing hydrogels as biomaterials is the protein-repellent properties of many hydrogels, polyethylene glycol being an example of one such hydrogel.[16] However, current research demonstrates that hydrogels have protein selective properties; in fact, the nature of the surface functionalities can determine the type of cells adhering to the surface of such engineered hydrogels.[17,18] The versatility in the chemistry of polymeric hydrogels and their consequent surface functionalities make them ideal candidates for reconstructive scaffolds. The gel-like properties of the ECM can be very well replicated by the polymeric hydrogel-based systems.[19–21] Hydrogels, by virtue of their hydrophilic, highly crosslinked character, have high permeability for oxygen, nutrients, and other water soluble metabolites.[22] Also, the physical shape of hydrogels can be easily manipulated to fit a particular defect or fracture site and the hydrogel architecture can be controlled to allow optimal diffusion and transport properties for vascularization of the bone tissue.[23]

Regardless of methods or materials used for the fabrication of biomimetic scaffolds, initial protein adsorption events determine much of the *in vivo* fate of the implanted scaffold. In particular, cell attachment and subsequent morphology and phenotype depend upon the initial proteins adsorbed on the scaffold. Therefore, by exploiting appropriate biomaterial–protein interactions and by transducing relevant signaling pathways, current biomedical research brings to the fore the use of biomaterials as morphogenetic guides, for finally integrating with targeted tissues *in vivo*.[7]

Recently, it has been reported that the incorporation of ionomers in polymeric structures can afford dramatic changes in the surface properties of biomaterials, greatly improving cell adhesion and proliferation *in vitro*.[17,18] Specifically, anionic scaffolds are being investigated as a novel class of biomaterials facilitating the morphogenetic processes for engineering tissue substitutes. The rationale for the use of phosphate and phosphonate-containing polymers stems from the mimicry of mineral bone matrix and bone-promoting proteins.[24] Further, biodegradable poly(phosphoester) ionomers afforded the capacity of modifying the side chain phosphate functionality and facilitating complexation with calcium ions, the latter being an attractive feature for transducing osteogenic cues.[25,26] In addition to the family of scaffolds with free phosphate groups, other anionic functionalities have been investigated for use as scaffolds for tissue engineering. Glycosaminoglycans (GAGs) and proteoglycans (PGs) contain significant sulfate modifications that interact directly with many receptors modulating important downstream pathways.[19] The conjectured model of proteoglycan-cell interaction is based on the structures and interactions of PGs and extracellular proteins.[27] Simply put, based on adhesive protein–PG interactions with cells, appropriate cell–matrix adhesion will allow the formation of tissues.[28,29] It is expected that similar reactions can be mimicked using synthetically derived polymers. Harnessing the diversity of ionomers available for investigation and exploiting the association of sulfates with biological macromolecules, researchers have tested the potential of sulfated forms of naturally occurring macromolecules for their biocompatibility. Fibronectin adsorbed onto sulfated hyaluronan substrates induced spreading of fibroblasts in a manner much improved compared to nonsulfated matrices.[30]

The elucidated role of naturally available and modified sulfated macromolecules in osteogenic tissue engineering prompted us to develop synthetic sulfonated scaffolds for assaying adhesion and proliferation of embryonic fibroblasts and human and calvarial preosteoblasts. To investigate the cytocompatibility of the synthetic sulfonated scaffolds, different proportions of a sulfonated monomer, sulfopropyl acrylate potassium (SPAK), were copolymerized with corresponding proportions of acrylamide (AM) to create differentially sulfonated hydrogel scaffolds. ECM mimicry was achieved via controlled presentation of serum proteins to cells by fabricated, sulfonated hydrogel scaffolds. The stochastic forces that govern the preferential alignment of macromolecules (serum proteins) for cell adhesion will be favored by the presence of a morphogenetic guide (sulfonated polymer). It is possible that the optimal number and arrangement of sulfonated groups in the polymeric hydrogel facilitate the favorable placement of the adhesive protein relative to the cell, thus generating appropriate cues for cell adhesion and consequently, migration and proliferation. With the introduction of an appropriate biodegradable crosslinker and the incorporation of various osteoinductive factors by derivatizing the sulfonate functionality constituting the fabricated scaffolds, these hydrogels appear to be promising candidates for osteogenic tissue engineering.

MATERIALS AND METHODS

Hydrogel fabrication

All chemicals were purchased from Sigma-Aldrich Chemical (St. Louis, MO) as ACS grade, or better, and

used without further purification unless otherwise specified. Hydrogels were formed from aqueous (double deionized water, DDI water) stock solutions of monomers, sulfopropyl acrylate potassium salt (SPAK), acrylamide (AM), acrylic acid (AA), and *N*,*N*′-methylene bisacrylamide (BIS), the initiator pair, ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylene diamine (TEMED), and surfactant, Pluronic® (PF127).[27,31,32] Aliquots of the stock solutions were mixed to create a final monomer and initiator feed. The total feed volumes of the SPAK-AM monomer mix, crosslinker, APS, and TEMED in the polymer forming solution were kept constant in all hydrogel formulations, with only the composition of AM and SPAK varying in each formulation. SPAK molar fractions (X_{SPAK}) in the monomer mixture were 0 (pSPAK0), 0.25 (pSPAK25), 0.50 (pSPAK50), 0.75 (pSPAK75), and 1.0 (pSPAK100); AM content made up the remainder of the monomer content ($X_{AM} = 1 - X_{SPAK}$). APS was freshly prepared each time the hydrogels were fabricated and added as the last component, just prior to vortexing. The reactants were then added to a mold consisting of two glass plates separated by a silicone rubber gasket (~5 mm) and allowed to polymerize overnight at room temperature. At the end of the reaction, thin sheets of hydrogel were removed from between the glass plates by removing the silicone or rubber spacers. The fabricated hydrogels obtained were then trimmed to size using 6 mm dermal biopsy punches. Next, the hydrogels were washed extensively with DDI water, followed by Dulbecco's phosphate buffered saline (DPBS) with penicillin (100 IU/mL) and streptomycin (1%) (pen/strep). This was done to leach out residual reactants and buffer the hydrogels to physiological pH values for cell culture experiments. For longer term storage, hydrogels were refrigerated in DPBS at 4°C. Hydrogels were handled under sterile conditions after fabrication for chemical assays, protein adsorption studies, and biocompatibility studies. Alternately, the hydrogels to be used for chemical analysis were leached of residual monomers over time using sterile filtered DDI water alone.

Chemical analysis

After a minimum of three 24-h washes in DDI water, small, circular pieces of hydrogel were cut, dehydrated, and sealed in vials for chemical analysis (Exeter Analytical, N. Chelmsford, MA, and ICP-MS; Perkin Elmer, Norwalk, CT). Results were compared with theoretical values, which were calculated based upon the monomer and crosslinker feed compositions in each hydrogel formulation.

Protein uptake

Protein uptake into hydrogels was examined by measuring the total amount of protein associated with equal diameter hydrogel disks (6 mm); thus, all hydrogels had equal surface area. Before protein uptake, the hydrogel disks were extensively washed with phosphate buffered saline (PBS) solutions and serum-free Dulbecco's modified Eagle's medium (DMEM) containing pen/strep for at least 2 h. Hydrogels were then allowed to swell in DMEM without serum in a humidified incubator with 5% CO_2 at 37°C for 24 h. Next, hydrogels were rinsed thrice with serum-free medium before being placed in 12-well plates with 2 mL DMEM supplemented with 10% fetal bovine serum (FBS). After 24 h in a humidified incubator, the hydrogels were transferred to a clean 12-well plate and gently rinsed with DPBS without calcium or magnesium for 10 s to remove loosely attached proteins. A 0.5-mL aliquot of 1% sodium dodecyl sulfate solution was pipetted onto each hydrogel, and the hydrogels were placed on a shaker for protein desorption for 30 min.[17] The concentration of proteins in the SDS solution was measured using the Micro BCA™ Protein Assay (Reagent Kit 23235, Pierce, Rockford, IL), as described by the manufacturer.[33] In addition, serum protein adsorption on tissue culture polystyrene (TCPS) was also measured for comparison.

Cell culture

NIH/3T3 mouse embryonic fibroblasts (3T3; ATCC CRL-1658™), MG-63 human osteosarcoma cells (MG-63; ATCC CRL-1427™),[34] and MC3T3-E1 subclone 4 mouse calvarial preosteoblasts (E1s4; ATCC CRL-2593™)[35] were cultured in DMEM supplemented with 10% bovine calf serum, minimum essential medium (Eagle's) (EMEM) with 10% fetal bovine serum, and alpha minimum essential medium (AMEM) without ascorbic acid with 10% fetal bovine serum, respectively. All cell types used in this study were maintained in 75-cm^2 tissue culture flasks in the appropriate culture media in a humidified atmosphere of 5% CO_2 at 37°C. After reaching around 75% confluence, cells were harvested by treatment with 0.05% trypsin (trypsinization). The cells were then replated at a density of 1×10^5 cells/mL (6.67×10^4 cells/cm^2), in 48-well tissue culture plates (BD Falcon™ Multiwell™ Culture Plates) on substrata consisting of the different pSPAK hydrogel compositions or TCPS (control). Cells growing on the different surfaces were then maintained in serum supplemented culture media for microscopy and assays at different time points.

Cell adhesion, proliferation, and morphology

Hydrogels used for all studies were conditioned in appropriate serum free media for at least 24 h followed by 2 h in appropriate serum supplemented media in order to condition the hydrogels with serum proteins while further buffering the hydrogels to physiological pH. This treatment was done just prior to seeding cells on the hydrogels. The control wells were also similarly treated. At specific times after seeding, the relative number of cells was determined using a modified MTT assay (CellTiter 96® AQueous One Solution Reagent; Promega Corporation, Madison, WI),[36] the MTS reagent was used as per the manufacturer's specifications with minor deviation. To avoid interference of the hydrogel with the reagent, cells were first released from the polymeric matrices by trypsin and then quantified using the MTS reagent. Trypsinization of cells was conducted with 100 μL of 0.05% trypsin/EDTA by incubating for around 5 min in a 37°C incubator and

gently striking the plate to dislodge the cells from the surface of the hydrogels. The trypsinized cells were then diluted with 500 μL of growth media and mixed using a 200-μL pipette tip without introducing bubbles.

Model cell lines were observed under a light microscope at different time points of culture and digitized images were taken at intervals with an Olympus IX70 phase contrast microscope and captured with a Retiga 1300 CCD camera. Further, with an objective to assay the phenotype of MC3T3-E1 subclone 4 mouse calvarial preosteoblasts after the addition of ascorbic acid supplemented differentiation media, the calvarial preosteoblasts were also photographed at various intervals, after adding AMEM (with 10% fetal bovine serum) supplemented with ascorbic acid, to induce differentiation of the preosteoblasts. All images were processed with IPLab software (BD Biosciences, Madison, WI).

Statistical analysis

A minimum of three samples were examined in all studies. All data is presented as a mean plus or minus (±) the standard deviation. Statistical analysis was performed using one-way ANOVA with post-hoc Student's *t*-test when appropriate. Differences at $p < 0.05$ were considered to be statistically significant. Finally, all error bars are presented as standard deviations.

RESULTS AND DISCUSSION

Fabrication and chemical analysis of hydrogels

Hydrogels were formed from all monomer compositions described. The qualitative structure of the hydrogels was similar immediately after production, but the hydrogels swelled to differing degrees depending upon the monomer composition (Fig. 1). Generally, the hydrogels, after monomer leaching, exhibited swelling characteristics as expected, with pSPAK0 swelling the least and pSPAK100 swelling the most. The increase in swelling of SPAK-AM hydrogels with increase in X_{SPAK} is due to charge repulsion between the pendant sulfonate groups in the hydrogel, which is the major driving force for swelling of ionic hydrogels.[37,38]

An underlying assumption of our further studies was based on the premise that increasing the molar feed fraction of SPAK would enhance the sulfonate content of the polymer and hence the biocompatibility of the scaffolds. Chemical analysis (sulfur-to-nitrogen ratio) in the differentially sulfonated hydrogels was found to be in good agreement with the theoretical expectations [Fig. 1(A)]. The estimate corresponding to the sulfur to nitrogen ratio for the pSPAK100 hydrogel formulation, theoretically, would tend to infinity because of the high ratio of sulfur relative to minimal nitrogen content. The small molar fraction of the added crosslinker, BIS, accounted for all of the nitrogen present in this formulation.

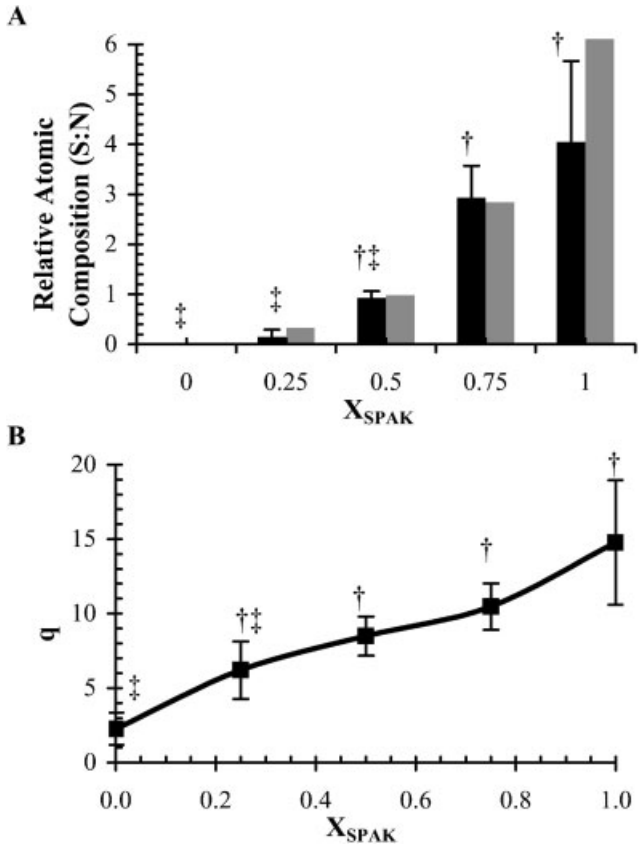

Figure 1. Correlation of (A) observed (black) and theoretical (grey) relative atomic composition (S:N) and (B) swelling ratio (q) with sulfopropyl acrylate content of the hydrogel (X_{SPAK}). Data are the average of three independent samples plus or minus the standard deviation (ave. ± SD). Statistical significant difference ($p < 0.05$) from pSPAK0 (†) and pSPAK100 (‡).

Serum protein uptake

Cell adhesion to a material, whether a naturally occurring material or a synthetic biomaterial, is mediated primarily by interaction between surface bound proteins and corresponding receptors on the cell membrane.[39] The goal of the evaluation of serum protein uptake by hydrogels with increasing proportion of sulfonate content was to test the hypothesis that increasing sulfonate content would lead to enhanced cell adhesion, proliferation, and differentiation, through increased and more biologically relevant protein absorption. Translating protein uptake to a complex *in vivo* scenario would call into play greater complexities of interfacial interaction, including the phenomenon widely known as the Vroman Effect.[40] In an attempt to replicate the biological system for preliminary *in vitro* studies, serum

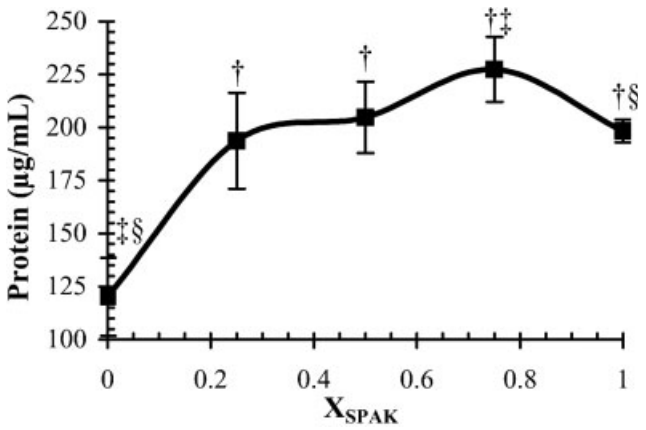

Figure 2. Correlation of serum protein uptake with sulfopropyl acrylate content of the hydrogel (X_{SPAK}). Data are the average of three independent samples plus or minus the standard deviation (ave. ± SD). Statistical significant difference ($p < 0.05$) from pSPAK0 (†), pSPAK100 (‡), and pSPAK75 (§).

supplemented DMEM was used to study protein uptake in a synchronous manner, rather than purifying and studying individual proteins.

A preliminary study of serum protein uptake by the differentially sulfonated hydrogels resulted in a biphasic behavior with the greatest protein adsorption at intermediate sulfonate (pSPAK) content (Fig. 2). The biphasic nature of protein adsorption could be attributed to a combination of factors.[41–43] Many proteins contain a combination of positively and negatively charged subdomains that interact with other proteins. Both highly electronegative and partially electronegative proteins will be excluded from highly negative hydrogels, while positively charged proteins alone will be attracted to the hydrogels as the pSPAK content increases. Since most serum proteins are electronegative, including albumin under physiological conditions, the bulk of the proteins will begin to be excluded from the hydrogels as pSPAK increases. However, increase in pSPAK will result in greater pore sizes associated with greater swelling, facilitating greater protein uptake by the hydrogels. Therefore, the combination of electrostatic and porosity alterations with change in pSPAK can account for the biphasic protein uptake curve.

Cell adhesion and proliferation

A common objective in the formulation of reconstructive implants is to engineer biomaterial niches that actively direct cell functions, especially adhesion, proliferation, and differentiation. It has been found that cell behavior on synthetic polymers is governed by several substratum properties, including surface chemistry and topography, surface hydrophilicity, ionic charge, and zeta potential. It might be expected that a substratum presenting an interface with hydrated anionic polymeric chains would repel cells due to steric hindrance. In fact, neutral and anionic hydrogels are often claimed to be "nonfouling", that is, rejecting proteins and cells, due to the steric hindrance effect of exposed polymer chains and electrostatically unfavorable interactions of cells with negatively charged polymeric surfaces.[44–46] However, water soluble sulfonated monomers such as styrene sulfonic acid sodium salt (anionic) grafted on nonionic surfaces exhibit high cell adhesion via protein adsorption.[47] Further, negatively charged phosphonic acid hydrogels support cellular activities.[17] On similar lines, we assayed the cell adhesive and proliferative properties of the fabricated sulfonated scaffolds with respect to TCPS, which served as a template for further studies on the fabricated polymeric matrices. The time-point of the assay was determined by attainment of ~75% confluence by pSPAK100 and TCPS (maximally proliferating cells), since anchorage-dependent cells that undergo contact inhibition may exhibit a slow change in the metabolic activity per cell at high densities.

Since adhesion is the first step toward tissue remodeling, the greater the favorable interaction of proteins with the associated substrate, the greater is the extent of cell adhesion. In both, a direct correlation was observed between the number of cells adherent (Fig. 3) and proliferating (Fig. 4) on the sulfonate-containing polymeric matrices. In general, it was noted that the pSPAK75 and pSPAK100 scaffolds demonstrated highest adhesion and proliferation of cells. Those with lower sulfonate contents resulted in balling up of cells, exhibiting dark clumps of celluloid-like material indicating incomplete cell attachment on the polymeric matrices. In general, pSPAK75 and pSPAK100 matrices appeared very similar to the TCPS control (Figs. 5–7).

The cellular compatibility of the cell lines tested was indicated by both the adhesion and proliferation trends. Comparing the cellular compatibility on the differentially sulfonated scaffolds with the protein adsorption by the scaffolds, there appeared to be a clear disparity between the trends. The protein adsorption data, interestingly, exhibited a biphasic trend. This clear disparity between cellular compatibility and protein adsorption was however not surprising. This is because cellular compatibility is a function of the extent as well as the nature of protein adsorption, specifically, the types of proteins and peptides adsorbed and the conformation of these adsorbed compounds.

Next, we examined the behavior of the fabricated hydrogels in the absence of serum proteins. Cells cultured in the absence of serum proteins did not

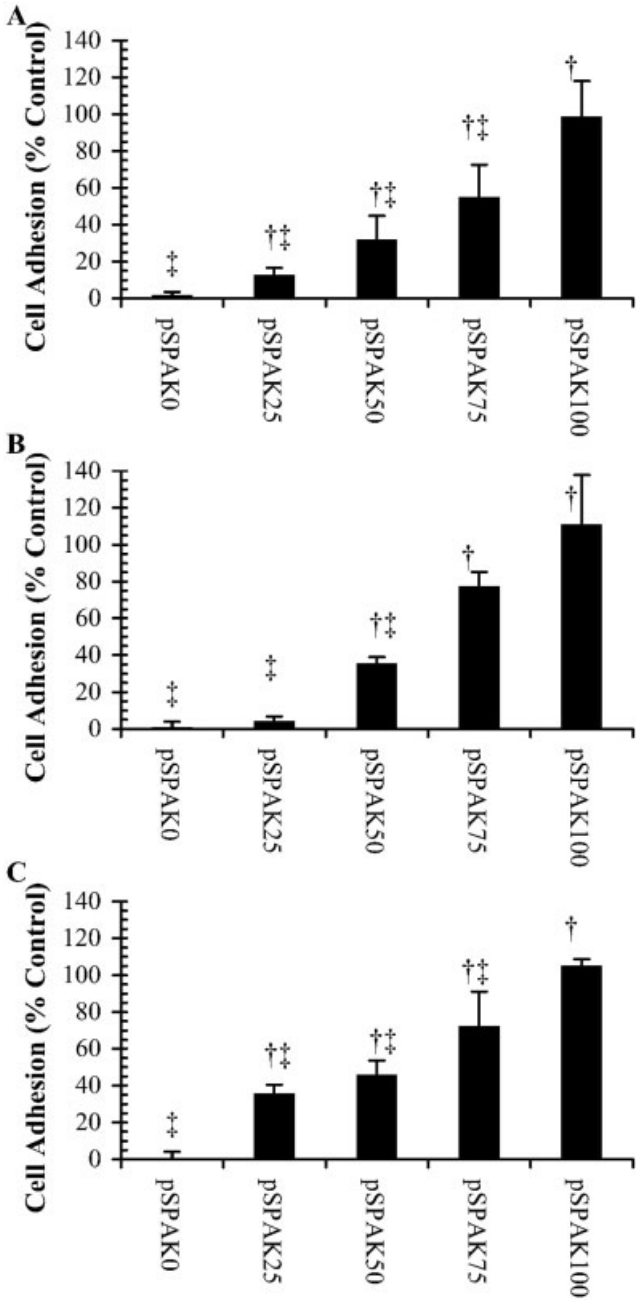

Figure 3. Cell adhesion for (A) 3T3, (B) MG-63, and (C) E1s4 cells as a function of SPAK content (X_{SPAK}) in hydrogels. Data are the average of three independent samples plus or minus the standard deviation (ave. ± SD). Statistical significant difference ($p < 0.05$) from pSPAK0 (†) and pSPAK100 (‡).

survive at the end of the second day of culture and resulted in cell counts tending to zero when assayed on the third day of culture. In contrast, in serum supplemented culture, the incorporation of anionic constituents into nonadhesive poly(acrylamide) hydrogels facilitated protein uptake, cell adhesion, and cell proliferation on the hydrogels. This confirmed the hypothesis that the sulfonated anionic groups induced greater adhesion and proliferation via favorable adsorption of serum proteins on the scaffolds.

CONCLUSIONS

Novel p(SPAK-*co*-AM) sulfonated hydrogels were synthesized to examine the *in vitro* biocompatibility

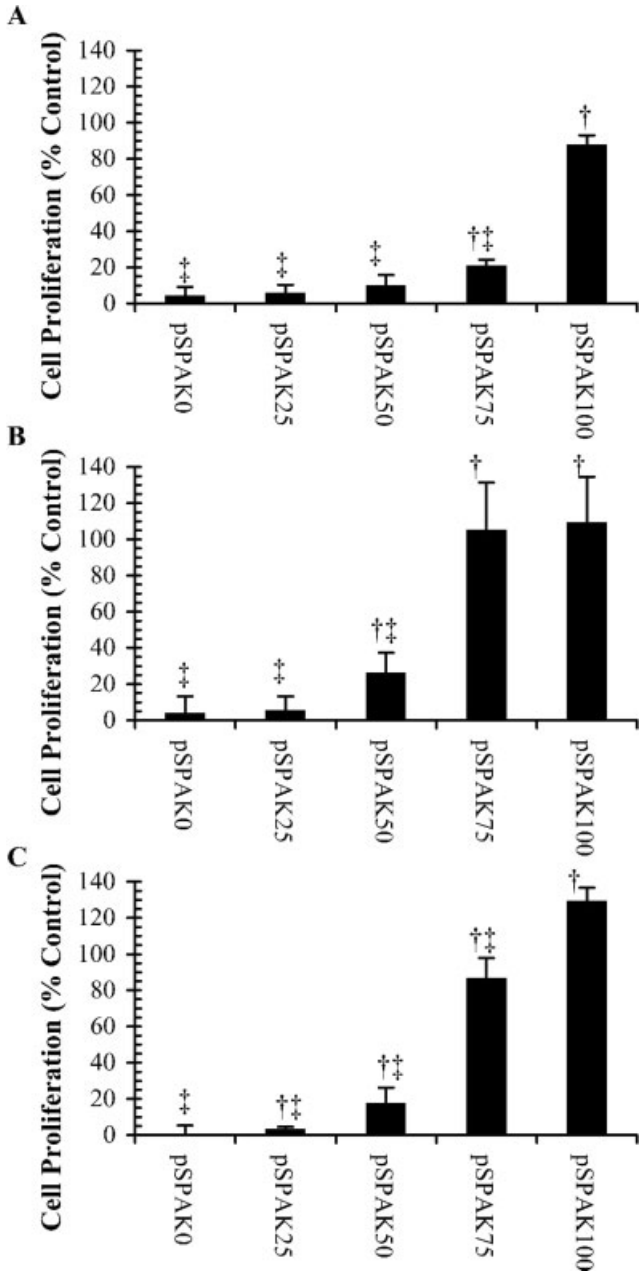

Figure 4. Cell proliferation for (A) 3T3 cells, (B) MG-63 cells, and (C) E1s4 cells as a function of SPAK content (X_{SPAK}) in hydrogels. Data are the average of three independent samples plus or minus the standard deviation (ave. ± SD). Statistical significant difference ($p < 0.05$) from pSPAK0 (†) and pSPAK100 (‡).

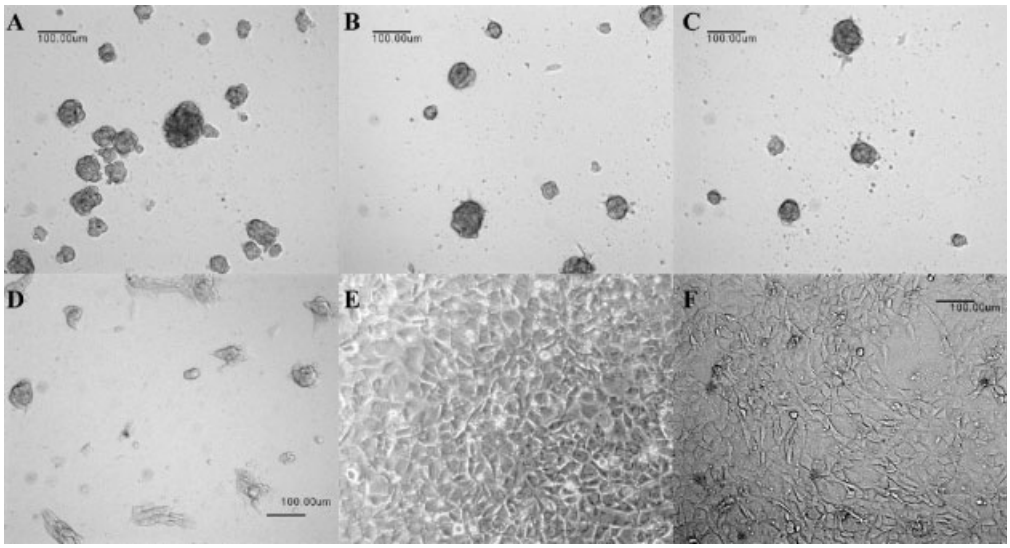

Figure 5. Representative 3T3 cell micrographs taken on the 4th day of culture while grown on (A) pSPAK0, (B) pSPAK25, (C) pSPAK50, (D) pSPAK75, (E) pSPAK100, and (F) TCPS (control) surfaces. All micrographs were taken at the same magnification and the scale bar is 100 μm.

of the fabricated hydrogels toward promoting osteogenesis. Importantly, from chemical analysis data, it was confirmed that the proportion of SPAK used in the fabrication of the SPAK-AM hydrogels had a significant correlation with the actual proportion of sulfonate incorporated in the polymeric hydrogels. Since fibroblasts play a major role in wound healing mechanisms,[48] the biocompatibility of the fabricated hydrogels with undifferentiated fibroblasts was tested, in addition to testing with osteoblast-like cells. The fabricated sulfonated hydrogels appeared to mimic sulfated GAGs abounding in the ECM via favorable adsorption of proteins from serum supplemented media. Adhesion and proliferation of the model cell lines were augmented in proportion to the hydrogel's sulfonate content, with pSPAK75 and pSPAK100 exhibiting promising potential toward osteogenic healing and reconstruction. In fact, pSPAK100 demonstrated comparable, or in some cases, higher rates of adhesion and proliferation of cells than the control-tissue culture treated commercial polystyrene. This study indicates the ability to modulate the adhesion and proliferation of osteoblast-like cells by altering the sulfonate content

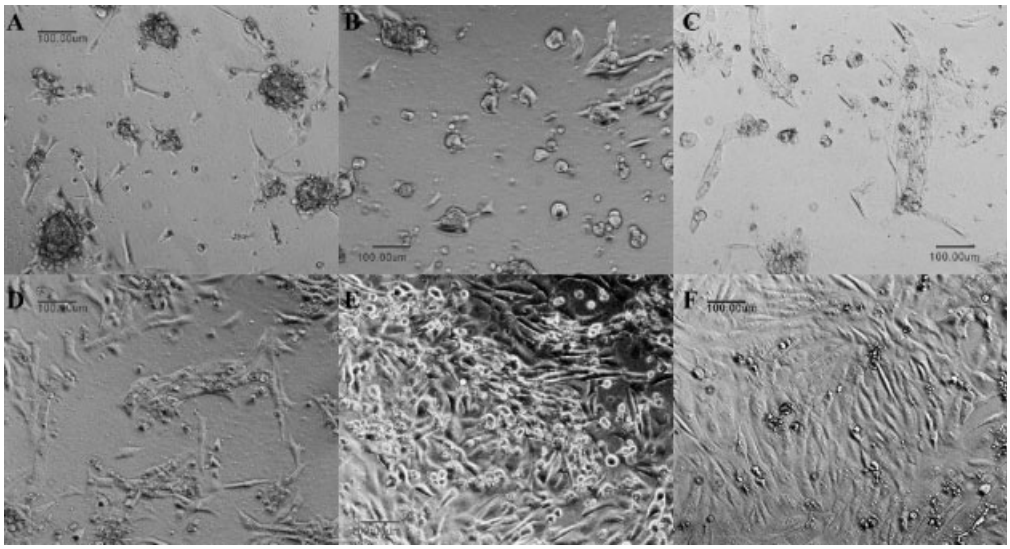

Figure 6. Representative MG-63 cell micrographs taken on the 4th day of culture while grown on (A) pSPAK0, (B) pSPAK25, (C) pSPAK50, (D) pSPAK75, (E) pSPAK100, and (F) TCPS (control) surfaces. All micrographs were taken at the same magnification and the scale bar is 100 μm.

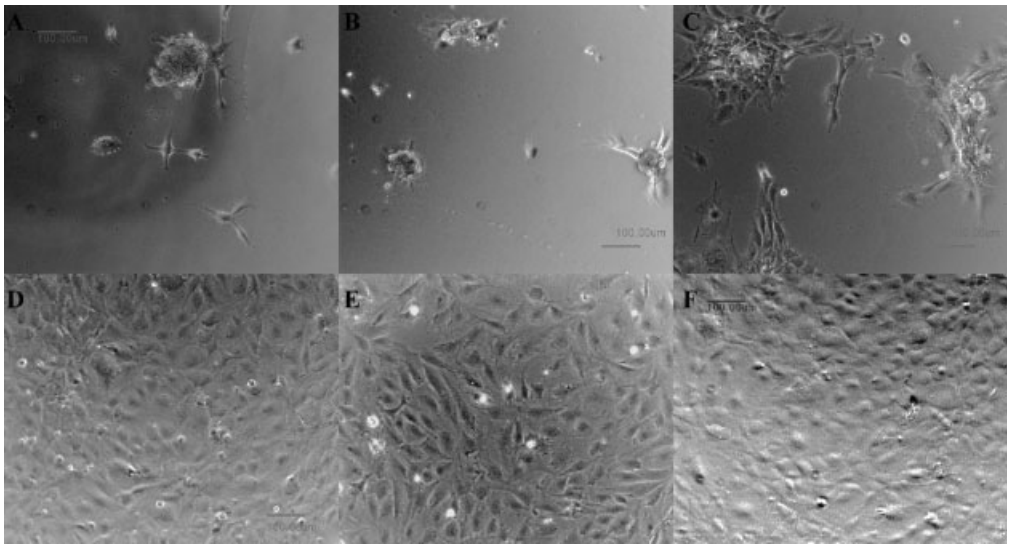

Figure 7. Representative E1s4 cell micrographs taken on the 4th day of culture while grown on (A) pSPAK0, (B) pSPAK25, (C) pSPAK50, (D) pSPAK75, (E) pSPAK100, and (F) TCPS (control) surfaces. All micrographs were taken at the same magnification and the scale bar is 100 μm.

of the hydrogels, and more generally by altering the anionic functionality of the hydrogel. The exact nature of the signal transduction mechanisms underlying the recruitment, migration, and proliferation of cells, however, remain to be unraveled.

Since biodegradable polymeric constructs are completely resorbed over time, leaving only newly formed tissue, a chimera of donor cells, and recipient mesenchymal elements, incorporating a biodegradable crosslinker in our fabricated polymeric matrices is projected to be an important future development.[49,50] Further, scaffolds for osteogenesis should mimic natural bone morphology and function to optimize osteointegration. Monitoring differentiation indices that quantify matrix production by osteoblastic lineages would constitute a logical next step to assay osteointegration of orthopedic prostheses. In future work, these *in vitro* investigations will be supplemented with determination of the *in vivo* toxicity and bone regenerative potential of biodegradable, sulfonated scaffolds in appropriate animal models.

Overall, the results from this study further testify to the potential of anionic hydrogels in the context of orthopedic engineering and open an exciting area of further research.

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