BTC: Fibrinogen, in tris-buffered saline (TBS), pH 7.8, and incubated for 20 minutes at 37° C. An equal volume of thrombin (Sigma-Aldrich, St. Louis, Mo.) in 40mM $CaCl_2$; and a final concentration of 100 U/ml was added and incubated for 10 minutes at 37° C. The resulting gels were then rinsed with TBS (pH 7.8) to remove unbound free BTC-PEG-BTC. To prepare ASC-seeded gels, cells of at different concentrations (5000-50000/gel) were added to PEG-fibrinogen mixture before the initiation of gelation with thrombin. The cell-gel mixture was placed in a 12 well plate and incubated at 37° C. for 10 minutes. After complete gelation, the PEGylated fibrin gels were washed twice with HBSS and incubated with α-MEM (alpha minimal essential media) supplemented with 10% FBS, antibiotic-antimycotic (100 U/ml of Penicillin G, 100 μg/ml streptomycin sulfate and 0.25 μg/ml Amphotericin B) and 2mM Lglutamine (Invitrogen, Carlsbad, Calif.), in a 5% CO2 humidified incubator at 37° C.

[0056] Morphological Analysis and Viability of ASC in PEGylated Fibrin Gel

[0057] ASC seeded at different concentrations (5000-50000 cells) in PEGylated fibrin gel were observed and photomicrographs were taken at different time points (1, 3, 5 and 7 days) using an Olympus IX71 inverted microscope equipped with reflected fluorescence system and DP71 digital camera (Olympus America Inc, Center Valley, Pa.). At similar time points growth media was removed and 50 μl MTT (3-(4,5-dimethylthiozole-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich, St. Louis, Mo.) solution (5 mg/ml) was added to each gel and incubated for 5 hours in a 5% CO2 humidified incubator at 37° C. After incubation the MTT solution was removed and 500 μl of isopropyl-acetone mixture (1:1) was added and allowed to incubate for 30 minutes to solubilize and extract the formazan complex. The gel-solvent mixture was then centrifuged at 2700×g for 10 minutes and the supernatant was collected and added to individual wells of a 24 well plate. Absorbance of the supernatants and isopropyl alcohol-acetone mixture (reagent blank) was measured at 570 nm with 630 nm as reference using Molecular Devices Spectramax M2 Microplate Reader (Molecular Devices, Sunnyvale, Calif.). The cell number associated with PEGylated fibrin gel was determined relative to the standard absorbance value obtained from known numbers of viable ASC.

[0058] Immunocytochemical Analysis of ASC Seeded PEGylated Fibrin Gels

[0059] Immunostaining of ASC in PEGylated fibrin gels. Before cryosectioning, gels (day 11) were cryopreserved using gradient sucrose cryopreservation technique. Briefly, the gels were washed with HBSS (twice, 5 minutes), fixed with 4% paraformaldehyde (PFA, EMS, Hatfield, Pa.) treated serially with increasing concentrations of sucrose (from 5% and 20%) and then incubated overnight with 20% sucrose at 4° C. The sucrose treated gels were embedded in a 20% Sucrose-HistoprepTM (Fisher, Pittsburgh, Pa.) mixture (2:1) and flash frozen. Sections, 10-12 μm thick, were cut using a cryostat (Leica Microsystems, Nussloch, GmbH), washed with sterile HBSS and fixed with 4% PFA for 20 minutes. Non specific Fc receptor mediated blocking sites were blocked by incubating the sections for 40 minutes-1hr with 1% BSA (bovine serum albumin) in HBSS containing 0.01% Triton X 100 and washed twice (5 minutes) with HBSS. To assess the endogenic immunophenotype, sections were stained with anti rat CD31 (PECAM-1, 8 μg, R&D Systems, Minneapolis, Minn.) and von Willebrand factor (vWF, 10 μg/ml, Millipore, Billerica, Mass.) specific monoclonal primary antibodies. For identifying pericyte immunophenotype, rat specific monoclonal antibodies specific to Chrondroitin sulfate proteoglycan (NG2, 20 μg/ml, Millipore, Billerica, Mass.), platelet derived growth factor receptor beta (PDGFRβ, 10 μg/ml, R&D Systems, Minneapolis, Minn.) and alpha smooth muscle actin (α-SMA, 8 μg/ml, Abcam, Cambridge, Mass.) antibodies were used. The sections were subjected to single or double immunofluorescent staining by incubating with a monoclonal primary antibody or mixture of two antibodies targeted towards two different antigens at 4° C. overnight. Following incubation of unconjugated primary labeled antibodies, sections were washed twice (5 minutes) with HBSS and incubated with 5 μg/ml host species specific Alexa fluor 488 and/or Alexa fluor 594 secondary antibodies (Invitrogen, Carlsbad, Calif.) for 45 minutes at 4° C. Finally, the sections were washed twice (5 minutes) and nuclei stained with Hoechst 33342 (Invitrogen, Carlsbad, Calif.). Non-specific fluorescence was determined using sections incubated with respective fluorophore labeled secondary antibodies.

[0060] Fluorescent and confocal microscopic morphological analysis. Epifluorescence of cells and gel sections were observed using Olympus IX71 inverted microscope equipped with reflected fluorescence system (Olympus America Inc, Center Valley, Pa.). Photomicrographs were taken using DP71 digital camera and image overlay was carried out using DP controller application software. Three-dimensional images, were taken using an Olympus FV-500 Laser Scanning Confocal Microscope (Olympus America Inc.), equipped with three channel detection system for fluorescence, a differential interference contrast image (DIC) laser light source and Zstepper motor. The three dimensional stereoscopic images and movie were generated from a series of Z-stacked photomicrographs around the Z-axis using Fluoview and Tiempo Ratio Imaging software and final images processed using Image J software (image processing and analysis in Java, NIH, Bethesda, Md.).

[0061] RNA Isolation and Real Time Polymerase Chain Reaction (RT-PCR)

[0062] Total RNA from ASC in PEGylated fibrin gel at 1,3,5,7 and 11 days were isolated using Trizol LS reagent (Invitrogen, Carlsbad, Calif., USA) with modifications. Gels were rinsed with HBSS and carefully removed from the culture well. Four gels from each time were pooled together, minced, 16 ml of Trizol LS reagent was added and incubated for 10-15 minutes in ice. Following incubation 8 ml of chloroform was added, mixed and the aqueous phase separated by centrifugation. The RNA was then purified using mini spin columns. The concentration and quality of the purified RNA was determined at OD260/280 using a NanoDrop spectrometer (Nanodrop Technologies, Inc., Wilmington, Del.). Complementary DNA was synthesized from 150ng of total RNA, in duplicate, using SuperScript™III first strand synthesis supermix with oligo-dT primers (Invitrogen, Carlsbad, Calif., USA). A control lacking the RNA sample was synthesized to detect the random production of cDNA through contaminants. Oligonucleotide primer sequences specific to endothelial (CD31 and vWF) and pericyte markers (NG2 and PDGFRβ) were purchased from SA Biosciences (Frederick, Md.). Master mixes containing 200 nM of forward and reverse primers with SYBR®GreenERT™, qPCR supermix (Invitrogen, Carlsbad, Calif.) and the synthesized cDNA were added to appropriate wells. RT-PCR was carried out using a Bio-Rad CFX96 thermal cycler system (Bio-Rad, Hercules,