Virginia Commonwealth University
VCU Scholars Compass

Theses and Dissertations | Graduate School

2008

ANGIOGENIC POTENTIAL OF HUMAN MACROPHAGES ON ELECTROSPUN BIORESORBABLE VASCULAR GRAFTS

Koyal Garg
Virginia Commonwealth University

Follow this and additional works at: https://scholarscompass.vcu.edu/etd

 Part of the Biomedical Engineering and Bioengineering Commons

© The Author

Downloaded from
https://scholarscompass.vcu.edu/etd/1652

This Thesis is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.

www.manaraa.com

Virginia Commonwealth University

This is to certify that the thesis prepared by Koyal Garg entitled Angiogenic Potential of Human Macrophages on Electrospun Bioreabsorbable Vascular Grafts has been approved by her committee as satisfactory completion of the thesis requirement for the degree of Master of Science in Biomedical Engineering.

Gary L. Bowlin, Ph.D., Director of Thesis, School of Engineering

David G. Simpson, Ph.D., School of Medicine

Hu Yang, Ph.D., School of Engineering

Gerald E. Miller, Ph.D., Chair of Biomedical Engineering, School of Engineering

Rosalyn S. Hobson, Ph.D., Associate Dean of Graduate Studies, School of Engineering

Russell D. Jamison, Ph.D., Dean, School of Engineering

F. Douglas Boudinot, Ph.D., Dean of the School of Graduate Studies

Date

www.manaraa.com

© Koyal Garg, 2008

All Rights Reserved

www.manaraa.com

ANGIOGENIC POTENTIAL OF HUMAN MACROPHAGES ON ELECTROSPUN BIORESORBABLE VASCULAR GRAFTS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering at Virginia Commonwealth University.

by

KOYAL GARG
B.E., Avinashilingam Deemed University, India, 2006

Director: GARY L. BOWLIN, PH.D.
ASSOCIATE PROFESSOR, BIOMEDICAL ENGINEERING

Virginia Commonwealth University
Richmond, Virginia
December, 2008

www.manaraa.com

Acknowledgement

I would like to start by thanking Dr. Bowlin, my advisor and mentor who gave me the opportunity to work in his lab. He has provided guidance and focus to both me and my research project. I am also thankful to his understanding, encouraging and appreciative attitude that kept me going. I have not worked closely with Dr. Simpson and Dr. Yang, but would like to thank you both for being on my committee and appreciate your support with my efforts to earn this Master's degree. I would also like to thank my lab members for putting up with my madness. Special thanks to Scott Sell and Parthasarathy Madurantakam, for patiently listening to me and providing me help and information. I learned a lot from both of you. Many thanks to Donna & Matt Smith whose support and help got me started on ELISAs. I would also like to thank Josh Grant and Catherine Barnes for letting me shadow them. Thanks also go to my labmates, including Yas Maghdouri, Anna Bulysheva, Michael P. Francis, Michael McClure and Patricia Wolfe for making the lab a comfortable and fun place to work in. Last but not the least; I would like to thank the most vital part of life, my family. I can never thank my loving and caring parents enough for the endless support and guidance that they have given me all these years. My late grandmother deserves a big portion of my thanks. She has been a tremendous source of strength and will always be in my heart. To my younger brother, thank you for being my reason to smile.

www.manaraa.com

Table of Contents

www.manaraa.com

www.manaraa.com

List of Figures

Page

www.manaraa.com

www.manaraa.com

Abstract

ANGIOGENIC POTENTIAL OF HUMAN MACROPHAGES ON ELECTROSPUN BIORESORBABLE VASCULAR GRAFTS

By KOYAL GARG, B.E.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering at Virginia Commonwealth University.

Virginia Commonwealth University, 2008

Major Director: Dr. Gary L. Bowlin
Associate Professor, Biomedical Engineering

The aim of this study was to investigate macrophage interactions with electrospun scaffolds and quantify the expression of vital angiogenic growth factors *in vitro*. This study will further help in evaluating the potential of these electrospun constructs as vascular grafts for tissue repair and regeneration *in situ*. Human peripheral blood macrophages were seeded in serum free media on electrospun (10 mm) discs of polydioxanone (PDO), elastin

www.manaraa.com

and PDO:elastin blends (50:50, 70:30 and 90:10). The growth factor secretion was analyzed by ELISA. Macrophages produced high levels of vascular endothelial growth factor (VEGF) and acidic fibroblast growth factor (aFGF). Transforming growth factor beta-1 (TGF-β1) secretion was relatively low and there was negligible production of basic fibroblast growth factor (bFGF). Histology revealed direct correlation between cell infiltration into scaffolds and the PDO concentration. There was greater macrophage infiltration through fibrous networks of the PDO and 90:10 scaffolds. Therefore, it can be anticipated that these scaffolds will support tissue regeneration and angiogenesis.

www.manaraa.com

INTRODUCTION

Macrophages are known to play a central role in implant healing, vascularization and integration into the native tissue. It has been established from previous studies that the fate of a tissue engineered implant depends upon the kind of macrophage response it elicits [1]. Tissue regeneration begins with phagocytosis of debris and extracellular matrix (ECM) remodeling and reorganization through enzymes released by the macrophage which include proteinase, collagenase, elastase and hyaluronidase [2]. The second step involves cell recruitment, migration, proliferation and ECM formation via the spectrum of cytokines produced by the macrophage. The third and final step of tissue regeneration is angiogenesis, a process by which new capillary blood vessels are generated from a pre-existing vascular system. Angiogenesis is fundamental to wound healing, reproduction and embryonic development [2]. It results from stimulation of endothelial cells, which causes them to proliferate and migrate towards the source of pro-angiogenic molecules. On reaching a certain appropriate density, junctions between endothelial cells are formed, components of ECM are produced and a new basal membrane is formed [3] . The endothelial cells then progress to form a hollow tube. All steps in this transformation are induced by the myriad of growth factors derived from the macrophage.

www.manaraa.com

Project Synopsis

The aim of this study was to investigate macrophage interactions with electrospun scaffolds and quantify the expression of four vital angiogenic growth factors *in vitro,* vascular endothelial growth factor (VEGF), acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF) and transforming growth factor beta-1 (TGF-β1). This study will further help in evaluating the potential of these electrospun constructs as vascular grafts for tissue repair and regeneration *in situ*.

Polydioxanone (PDO) and elastin were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol in concentrations of 100 mg/ml and 200 mg/ml respectively. These solutions were blended in the ratios of 50:50, 70:30 and 90:10 (PDO:elastin) by volume. Pure PDO (100:0) and pure elastin (0:100) were also used in the study. These were then electrospun into a flat sheet.

Human peripheral blood monocytes (CRL9855, ATCC) were seeded into culture at a density of $6x10^7$ cells per 75 cm^2 flask in Dulbecco's modified eagle medium (high glucose) with 20% heat inactivated fetal bovine serum and 10% heat inactivated AB+ serum. This cell concentration provided a high seeding density for differentiation of monocytes to macrophages. Macrophages were obtained after 21 days of culture. The macrophages were then seeded at a density of 400,000 cells/well on electrospun (10 mm) disks of PDO, elastin, PDO:elastin blends (50:50, 70:30 and 90:10) and on tissue culture plastic (TCP) in a 48 well plate. The supernatants were collected on day 7, 14, 21 and 28 and stored at -70°C until analyzed by ELISA.

www.manaraa.com

BACKGROUND INFORMATION

Blood Vessel Architecture

The complex blood vessel system of the human body serves two main functions, delivery of oxygen and nutrients to the tissues and removal of metabolites for clearance or re-oxygenation. As shown in figure 1, the artery is composed of three different layers or tunics of different compositions which surround a hollow core or lumen through which the blood flows. The innermost layer, surrounding the lumen is known as the intima and is made up of single layer of endothelial cells on a basement membrane rich in collagen IV and elastin. This layer contacts the bloodstream and endothelial cells provide a crucial barrier to platelet activation by secreting specific molecules like nitric oxide and prevent thrombus formation. The thick middle layer, the media, is composed of several layers of smooth muscle cells in an extracellular matrix of collagen types I and III, elastin and proteoglycans. In elastic arteries like the aorta, the elastic lamellae allow the artery to maintain sufficient blood pressure with variations in hemodynamic stress of the cardiac systole and diastole. In muscular arteries, elastin is assembled as fibers. The outermost adventitial layer is composed of fibroblasts and randomly arranged collagen type I. This, collagen provides tensile support and prevents vessel rupture. The proteoglycans contribute

www.manaraa.com

to the compressibility and the elastin fibers give elasticity to the vessel and provide the ability to recover from pulsatile deformations. Its elastic nature dominates the low strain mechanical response of the vessel to blood flow and prevents pulsatile energy from being dissipated as heat [4, 5].

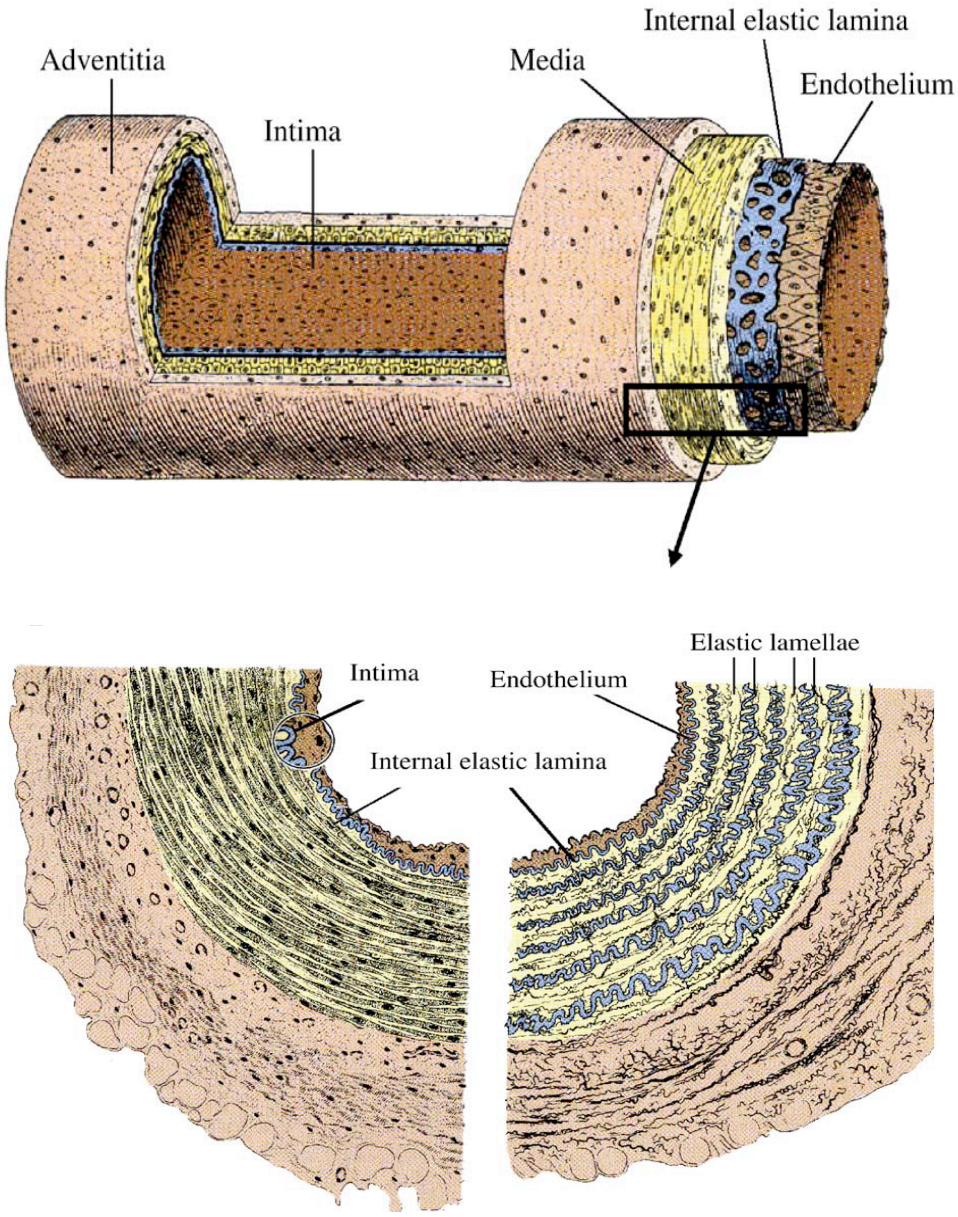


Fig. 1. Blood vessel architecture [6].

www.manaraa.com

The Macrophage

Macrophages belong to the mononuclear phagocyte system. This system comprises the bone marrow monoblasts, pro-monocytes, peripheral blood monocytes and tissue macrophages. They share a common progenitor cell with granulocytes in the bone marrow. This common progenitor is called the colony-forming unit, granulocyte-macrophage (CFU-GM) because of its ability to give rise to colonies of monocytes and neutrophils in a semi-solid marrow cultures. When monocytes enter the blood stream and migrate into tissues, they undergo final differentiation to tissue macrophages (Fig.2). Macrophages are present ubiquitously in all tissues and display great structural and functional heterogeneity as histiocytes, alveolar macrophages (lungs), kupffer cells (liver), osteoclasts (bone), peritoneal macrophages (peritoneum), synovia type A cells (synovium), or microglia (central nervous system). Macrophages are set into different functional states by a process known as activation. Activation of macrophages entails increased phagocytic or microbicidal activities and alteration of their chemotactic response and secretory activities. Activation is a complex process involving different stimuli. Macrophages increase performance of some functions and down regulate others. Some of the listed activators in literature are concanavalin A, endotoxin, lipopolysacchride and various cytokines such as interferon-γ (IFN- γ), granulocyte-macrophage colony stimulating factor (GM-CSF), platelet-activating factor (PAF) or monocyte chemotactic protein [7, 8].

www.manaraa.com

Macrophages also secrete a repertoire of proteases and growth factors such as bFGF, GM-CSF, transforming growth factor- alpha (TGF-α), TGF-β, Insulin-like growth factor-I (IGF-I), Platelet-derived growth factor (PDGF), VEGF, and other monokines such as inerleukins (IL-1, IL-6, IL-8), tumor necrosis factor-alpha (TNF- α), substance P, prostaglandins, interferons, thrombospondin 1 [7].

www.manaraa.com

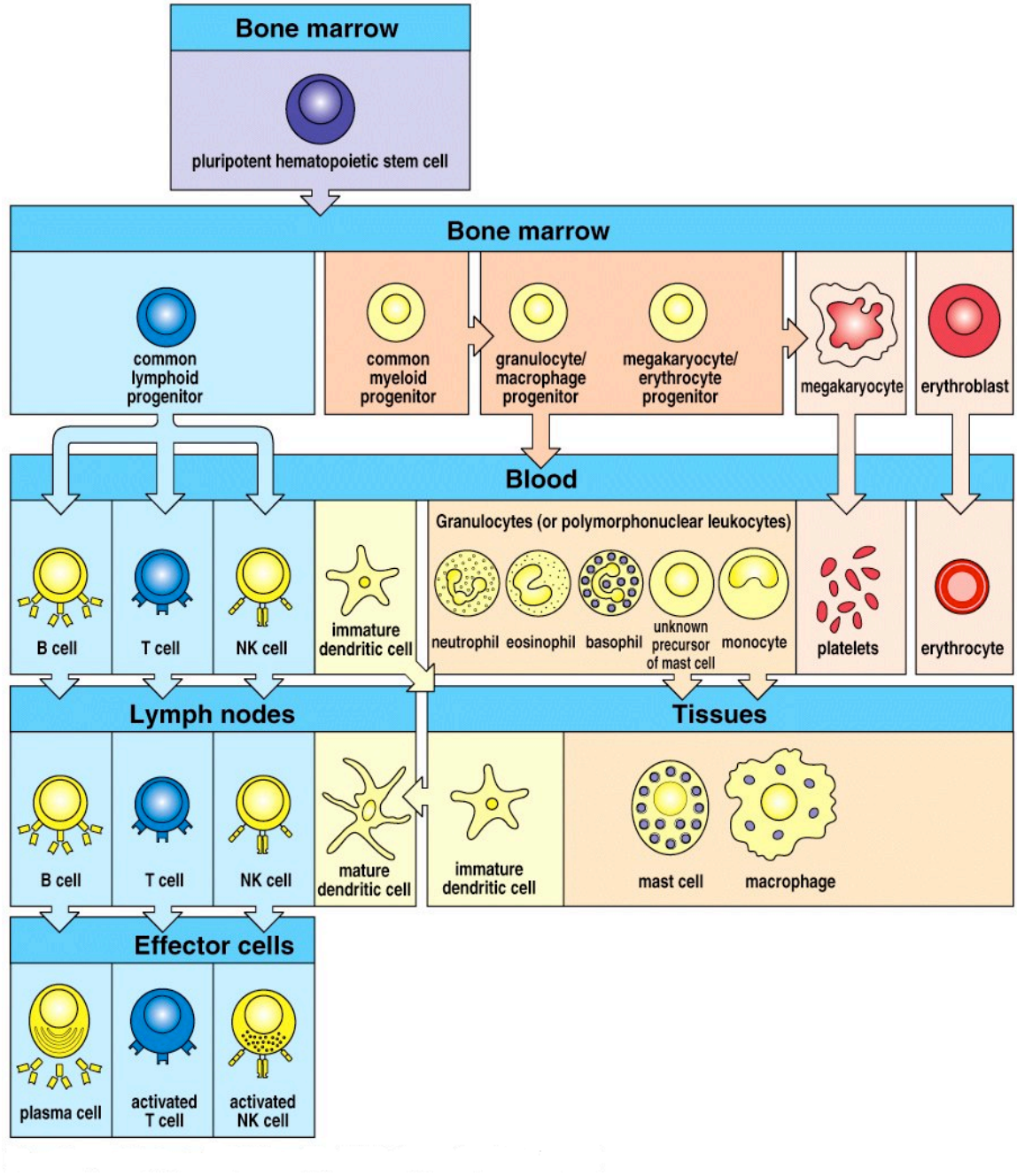
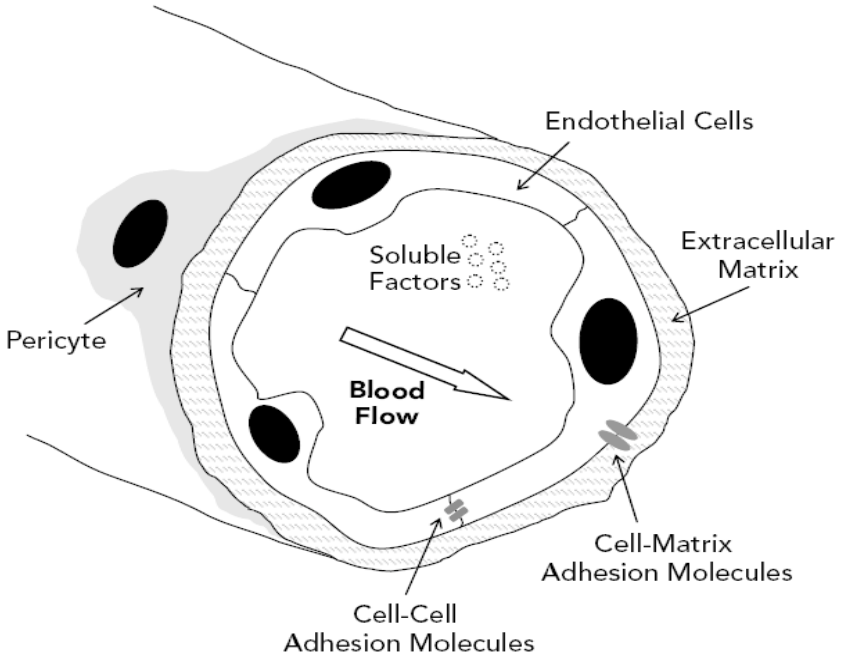


Fig. 2. The mononuclear-phagocyte system [9].

www.manaraa.com

Macrophages and Angiogenesis

The macrophage is the major differentiated cell of the mononuclear phagocyte system. It originates in the bone marrow as an immature monocyte. After entering the peripheral blood, monocytes are recruited by chemokines into the tissue and undergo differentiation into macrophages. The specialization and activation of these cells are mainly influenced by local stimuli [10, 11]. Angiogenesis is a multi-step process involving various cell types. It is tightly regulated by several microenvironmental factors in the blood vessel, including soluble molecules (e.g., growth factors and cytokines), extracellular matrices (ECM), interactions between adjacent endothelial cells (ECs) and ECs with other cell types, as well as mechanical forces originating from ECs themselves, blood flow, and extravascular tissue activity (Fig.3).



www.manaraa.com

Fig.3 Microenvironmental cues that affect angiogenesis [12].

The outstanding role of macrophages in angiogenesis can be attributed to their much longer half life as compared to granulocytes and platelets and secretion of myriad of growth factors for initiation, maintenance and termination of the angiogenic process (Fig.4). Also, macrophages are resident in all tissues in greater numbers than other blood borne cells and the distinct subtypes can always be recruited from the blood stream. They have heterogeneous functionality and can be activated from an inactive non-angiogenic stage to an angiogenic stage [7]. Growth factors are released by macrophages only when they are activated. The authors speculate that the macrophages will get activated when they come in contact with the biomaterials [13]. There are two known phenotypes of macrophage activation. Classically activated macrophages up regulate pro-inflammatory cytokines, inhibit anti-inflammatory cytokines and fibrogenesis. On the other hand, alternatively activated macrophages inhibit pro-inflammatory cytokines, promote anti-inflammatory cytokines and enhance fibrogenesis. The biomaterial adherent macrophage phenotype is known to have a different cytokine profile than either classically or alternatively activated macrophages indicating that the biomaterial activation is unique. Macrophage activation and cytokine expression can be modulated by the material properties, such as surface chemistry and surface topography [14].

www.manaraa.com

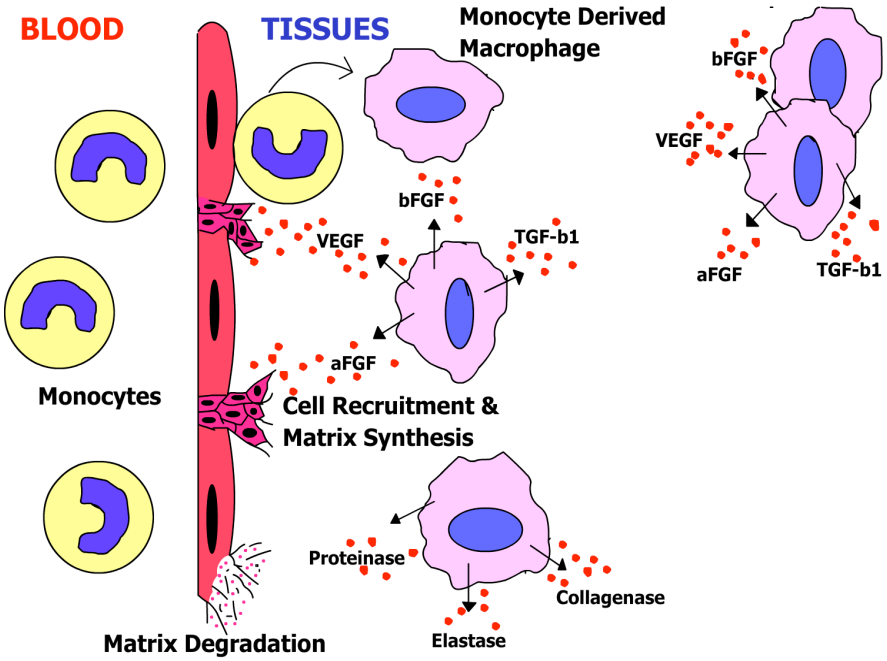


Fig. 4. Role of macrophages in angiogenesis.

Vital Angiogenic Molecules

The molecule VEGF (previously known as Vascular Permeability Factor) is by far the most potent angiogenic growth factor known. It is secreted in biologically active form by activated peripheral blood monocytes/macrophages. Besides directly stimulating angiogenesis, it also helps in regulating the production of proteases and their inhibitors, and promotes endothelial cell proliferation, migration, differentiation and survival [1]. VEGF induces vascular dilation and exudation of fibrin. The deposition of fibrin induces monocyte migration, penetration and tunnel formation in this new ECM [12]. bFGF and

www.manaraa.com

aFGF belong to a family of heparin binding (fibroblast) growth factors and can promote almost every phase of the angiogenesis cascade. They both induce urokinase-type plasminogen activator (uPA) in different cell types [3] . Secreted uPA converts plasminogen into plasmin, a protease which degrades ECM proteins and activates other metalloproteinases (MMPs) necessary for further degradation and remodeling of ECM . It also stimulates proliferation, DNA synthesis and migration of endothelial cells towards the place where new blood supply is needed [3] . Unlike VEGF, the actions of bFGF and aFGF are not limited to ECs. They act on most cells derived from mesoderm and ectoderm such as fibroblasts, pericytes and ECs.

TGF-β1 has both stimulatory and inhibitory effects on angiogenesis. It stimulates *in vivo* angiogenesis in presence of inflammatory response but is an inhibitor of endothelial cell growth *in vitro* [12, 15]. Actions of TGF-β1 depend upon its concentration and ECM organization. Higher concentration and two dimensional culture systems inhibited the migration and proliferation of endothelial cells whereas low concentration and three dimensional culture systems supported the proliferation and tube formation of endothelial cells [7]. It was also found that TGF-β1 mediates the inhibition of endothelial cell proliferation upon endothelial cell-pericyte contact in a coculture system. These effects are critical in stabilizing nascent, immature blood vessels [12]. Over expression of this cytokine results in fibrotic conditions since it promotes accumulation of ECM molecules [15].

www.manaraa.com

Tissue macrophages also express Angiopoietin-1 (Ang-1). It binds to the Tie-2 receptor and potently induces network formation, chemotactic response and survival during apoptosis. It also stabilizes nascent vessels by tightening the interaction between endothelial and periendothelial cells. It also causes sprouting of endothelial cells in fibrin gel but does not stimulate proliferation. As an antagonist of Ang-1, Angiopoietin-2 (Ang-2) competes with Ang-1 for binding of Tie-2 and blocks vessel stabilization from Tie-2 signaling. It thus loosens the interactions of ECs with pericytes and the ECM leading to vessel destabilization. Many different cell types, including ECs, fibroblasts, macrophages and platelets release a molecule called platelet-derived growth factor (PDGF). In blood vessel walls, PDGF receptors can be found in smooth muscle cells (SMCs), ECs and pericytes. In vivo studies have demonstrated that proliferation and migration of pericytes along angiogenic sprouts is mediated by PDGF [12].

Biomaterials for Vascular Tissue Engineering

The ideal vascular prosthetic must have the following performance characteristics: ease of handling, suture retention, ease of suture replacement, flexibility with kink resistance, biocompatibility, durability after implanatation and association with surrounding connective tissue, compliance matching that of the native artery, and resistance to aneurysm formation. Also, the graft must be easily manufactured, economical, easily stored and available in a variety of sizes [16].

www.manaraa.com

Initial graft research focused on Poly(ehtylene terephthalate) (Dacron) and expanded poly(tetrafluoroethylene) (e-PTFE) as prosthetic vascular grafts. Both Dacron and e-PTFE react with blood components and perigraft tissues in both advantageous and injurious ways. It has been documented that monocytes and macrophages produce cytokines such as interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) when incubated with Dacron and e-PTFE [17].

However, these prostheses remained permanently within the host after implantation. The concept of a slowly absorbable graft that could stimulate rapid and controlled regenerative process producing a "neoartery" was first described by Wesolowski et al. [18] and Ruderman et al. [19]. These grafts were partially bioresorbable and composed of Dacron and polylactide yarns. Bowland et al. described the use of Vicryl (a copolymer of polyglycolide and polylactide) as a fully bioresorbable vascular graft [20]. These early grafts were susceptible to aneurismal rupture and dilation.

Greisler et al. evaluated woven poly(glycolic acid) (PGA) grafts in a rabbit model. Four weeks after implantation, these grafts were shown to contain an inner capsule composed of a confluent layer of endothelial cells and smooth muscle like myofibroblasts in the midst of dense collagen fibers. Similarly constructed and implanted Dacron grafts failed to show the results described above [21, 22].

It is also important for the bioresorbable graft to regenerate a complex tissue of sufficient strength before prosthetic degradation so as to minimize the risk of aneurismal dilation. To circumvent this problem two or more bioresorbable materials with different

www.manaraa.com

resorption rates can be combined so that the more rapidly absorbed material induces rapid tissue ingrowth while the second material provides structural support [23]. Such composite grafts containing 69% polyglactin 910 (PG910) and 31% polypropylene were implanted into rabbit and dog arteries [24]. These grafts demonstrated 100% 1-year patency with no aneurysms. In another study, composite grafts of 74% PG910 and 26% polydioxanone (PDO/PDS) also showed 100% patency with no aneurysms one year after implantation [25]. PDO is a colorless, crystalline, biodegradable polymer that was developed for wound closure sutures. It exhibits high flexibility, higher strength retention, slower absorption rate, and lower inflammatory response as compared to poly(glycolide lactide) and poly(glycolic acid) [16]. Greisler et al. published results utilizing PDO absorbable vascular prosthetics in a rabbit aortic model of regeneration. The results showed no perigraft hematomas and the myofibroblast migration paralleled the macrophage–mediated degradation of the PDO structure. A confluent EC lining was present within two weeks, with the compliance of the explants at 1 year resembling an artery [26]. In another study, Geisler et al. demonstrated that PDO grafts had the highest production ratio of PGI_2/TxA_2 at a seven fold increase versus PGA and approximately equal to the native aorta control. The study showed a tissue dependence response in terms of thrombogenicity and the grafts containing PDO were less thrombogenic [27].

Recent advances in tissue engineering have shifted focus on synthetic and natural blends of polymers for use as vascular grafts. The introduction of natural polymer increases the hydrophilicity and cellular affinity of the material. This in turn enhances the

www.manaraa.com

cell attachment, migration and proliferation. The natural polymer invariably degrades first, creating more space for cell migration and tissue regeneration while the synthetic polymer maintains the structural integrity. Zhang et al. created gelatin and PCL electrospun blends that exhibited enhanced mechanical properties and better wettability than that obtained from PCL or gelatin alone [28]. A mixture of heparin and poly (ethylene glycol) (PEG) was electrospun for potential use as a wound dressing. The presence of PEG slowed down the release of heparin and permitted better biological outcomes [29]. Boland et al. demonstrated the electrospinning of a blend of collagen and elastin for use as vascular tissue engineered constructs [30].

In elastic tissues, polymers of elastin confer elasticity to the vessel and provide the ability to recover from pulsatile deformations [30]. Elastin is an abundant protein component of the native arterial tissue and is chosen to confer elasticity and bioactivity to the vascular prosthetic [5]. In arteries, elastin dictates tissue mechanics at low strains before stiffer collagen fibers are engaged. Elastin also prevents dynamic tissue creep by stretching under load and recoiling to their original configurations after the load is released. It is also a strong autocrine regulator of vascular smooth cells activity and this is crucial for prevention of fibrocellular pathology. Elastin induces actin stress fiber organization, inhibits smooth muscle cell proliferation, and regulates migration via a non-integrin heterotrimeric G protein coupled signaling pathway. In the absence of extracellular elastin, smooth muscle cell proliferation stenoses arteries. Therefore, in order to ensure

www.manaraa.com

proper mechanical function of the vessel and to prevent vessel stenosis, tissue engineered vascular implants must include an elastic component [6].

In the field of vascular tissue engineering and vascular prosthetic it is important to match or mimic the mechanical properties of the native tissue to eliminate mismatches between the prosthetic and the native tissue. Previous studies conducted on PDO: elastin blends have shown a range of mechanical properties that included those of native arterial tissue. The mechanical properties of the 50:50 blend closely matched the range of femoral artery values in almost every instance, the only exception being that the electrospun 50:50 blend exhibited higher ultimate stresses than the native vessel. Compliance is another important property of the blood vessels that assists in pushing blood through the circulation and prevents flow stagnation and graft thrombosis. It was found that the grafts containing elastin had greater compliance than pure PDO grafts [5].

The biomaterials chosen for this study were PDO blended with elastin in three ratios (50:50, 70:30, 90:10) to create nanofibrous, seamless tubular constructs for potential use as acellular vascular prosthetics [5]. This study will further help in evaluating the capability of this vascular prosthetic to promote *in situ* arterial tissue regeneration. To evaluate the effect of fiber diameter on macrophage interactions with biomaterials, different concentration of PDO (60, 80, 100, 120, 140mg/ml) were also electrospun and tested. The process used for the fabrication of this construct is electrospinning. Briefly, it consists of a charged polymer solution separated from an oppositely charged target by a set distance (air gap) to create a static electric field. When the electrostatic forces within the

www.manaraa.com

solution overcome the surface tension of the solution, a fine jet of entangled polymer chains is drawn out. The solvent evaporates as the jet travels through the air gap, and a dry fiber is collected on the target. The translation/rotation of the target ensures uniformity in structural characteristics [5].

The growth factors are released by macrophages only when they are activated. The authors speculate that the macrophages will be activated when they come in contact with the biomaterials. Macrophage activation through stimulation by lipopolysaccharide (LPS) or concanavalin A has been done in previous studies. It has been observed that the presence of such agents not only proportionately increase all the growth factor levels, but also manipulate the types being expressed leading to misinterpretation of the data. Therefore, in these studies we refrained from using stimulating agents to assess the growth factor production [31].

Host Response to Biomaterials

Following tissue injury, the normal healing response is initiated through a series of intricate events that include acute inflammation, the formation of granulation tissue, and eventual scar formation. The immediate response is to deluge the injured area with blood. Cleavage of fibrinogen within the blood into fibrin is done to form a blood clot that promotes platelet adhesion and aggregation. A spectrum of cytokines and growth factors are released to recruit white blood cells, primarily neutrophils. Monocytes are then

www.manaraa.com

recruited to the wound site where they differentiate into macrophages. The macrophages are responsible for cleaning up the wound site, by phagocytosis of debris which may include foreign material, bacteria, necrotic and apoptotic cells. In addition, they are also required for recruiting cells such as fibroblasts and endothelial cells. These cells convert the fibrin clot into a highly vascularized granulation tissue. The formation of blood vessels is crucial to the healing wound. Fibroblasts subsequently replace the granulation tissue by an extracellular matrix (ECM). The degree of ECM remodeling is dependant on the extent and location of the injury. It has been observed that in some cases, complete restoration of the tissue architecture is possible but mostly granulation tissue is remodeled into scar tissue.

When a biomaterial is implanted into the body it induces a different response, termed the foreign body reaction (Fig.5 and 6). Briefly, a biomaterial elicits nonspecific protein adsorption immediately upon implantation. Many different proteins adsorb to the surface in a range of conformations from native to denatured. However, non-specific protein adsorption has never been observed in the normal physiological process of wound healing. Thus, nonspecific protein adsorption is believed to be an instigator in the foreign body reaction. A variety of cells, that are key players in normal wound healing (such as monocytes, leukocytes, and platelets) adhere to these biomaterial surfaces and as a result may lead to upregulation of cytokines and subsequent proinflammatory mechanisms.

www.manaraa.com

Macrophage response to biomaterials is dependent on the size of the materials. Material particle sizes smaller than a single-nucleated macrophage (normally around 10 μm in diameter) can be phagocytozed by macrophages. Large particles (between 10 μm and several hundred micrometres) that are beyond the capacity of macrophage phagocytosis may be engulfed by multinucleated giant cells or foreign body giant cells [2]. Since the implant is significantly larger than the adhered macrophages, it prevents them from phagocytosing the foreign body. This initiates the chronic inflammation at the biomaterial interface and is followed by the fusion of the frustrated macrophages to form multinucleated foreign body giant cells that often persist for the lifetime of the implant. The resolution of macrophage responses to biomaterials depends on whether the material is degradable or nondegradable. Degradable materials are usually degraded by phagocytosis, or eroded via extracellular resorption, with or without the involvement of foreign body giant cells. Any associated inflammation is taken care of after total resorption of the biodegradable materials. Nondegradable materials cannot be degraded either within the macrophage phagosome, or by extracellular resorption. Macrophages infiltrate constantly to phagocytoze undigested particles or to fuse into foreign body giant cells and persist on the surface of the implanted materials. Therefore, for non-degradable biomaterials, it is vital to choose those that cause less macrophage responses for long-term implantation.

In the final stage of the foreign body reaction, the device is covered by an avascular, collagenous fibrous tissue that is usually 50–200 μm thick [32, 33].

www.manaraa.com

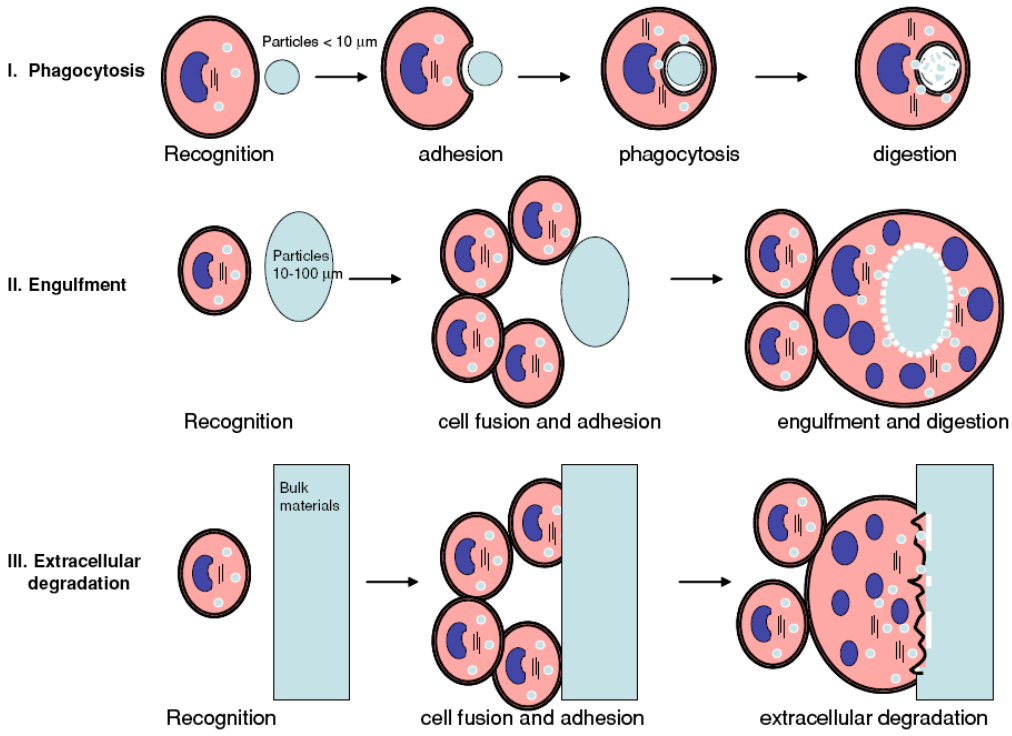


Fig. 5. Diagram showing macrophage response to biomaterials [2].

www.manaraa.com

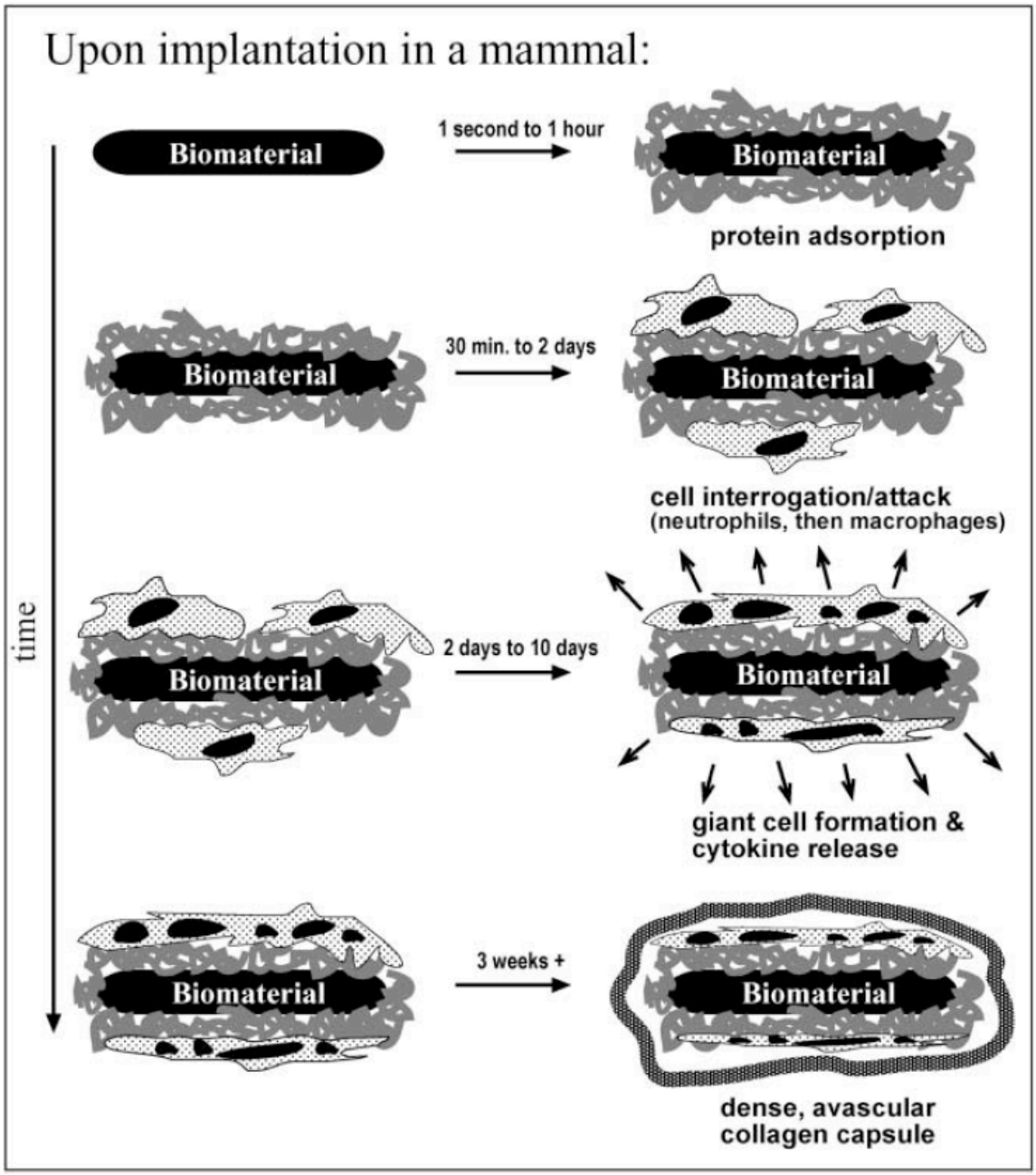


Fig. 6. Foreign body reaction to an implanted synthetic biomaterial [33].

www.manaraa.com

Electrospinning

A number of processing techniques such as drawing, template synthesis, phase separation, self assembly and electrospinning have been utilized to synthesize polymer nanofibers for tissue engineering. Drawing is a process that can make one-by-one very long single nanofibers. However, only a viscoelastic material that can undergo very strong deformations can be made into nanofibers through drawing. Template synthesis, uses a nanoporous membrane as a template to make nanofibers of a solid or hollow shape. A major drawback of the method is the inability to synthesize one-by-one continuous nanofibers. The phase separation method consists of dissolution, gelation, extraction using a different solvent, followed by freezing and drying. In self assembly procedure, pre-existing, individual components organize themselves into desired patterns and functions. However, both phase separation and self assembly processes are very time consuming in processing continuous polymer nanofibers. Electrospinning is an advantageous processing method for synthesizing one-by-one continuous nanofibers from various different polymers [34]. It's also a very simple, straight forward, cost-effective method to generate different types of scaffolds. Briefly, it consists of a charged polymer solution separated from an oppositely charged target by a set distance (air gap) to create a static electric field (Fig.7). When the electrostatic forces within the solution overcome the surface tension of the solution, a fine jet of entangled polymer chains is drawn out. The solvent evaporates as

www.manaraa.com

the jet travels through the air gap, and a dry fiber is collected on the target. The translation/rotation of the target ensures uniformity in structural characteristics [5] .

There are great advantages of using electrospinning for tissue engineering applications. Electrospinning is capable of producing extremely thin fibers with diameters ranging from microns down to few nanometers. In native tissues, cells are typically about one to two orders of magnitude bigger than the structural ECM proteins (50-300 nm). This allows the cells to be in direct contact with many ECM fibers. Therefore, the small-size fibers mimic the structural dimension of the ECM of several native tissues which are deposited and characterized by well-organized hierarchical fibrous structures realigning from nanometer to millimeter scale. The scaffolds produced by electrospinning possess a highly porous microstructure with interconnected pores and extremely high surface-area-to-volume ratio, which is conducive to tissue growth. In addition, it is a very versatile technique and allows the use of a variety of polymers, blends of different polymers, and inorganic materials. Recent advances in electrospinning have shown that integration of various substances such as additives, biomolecules, and living cells is possible in scaffolds for tailoring different application requirements [28].

www.manaraa.com

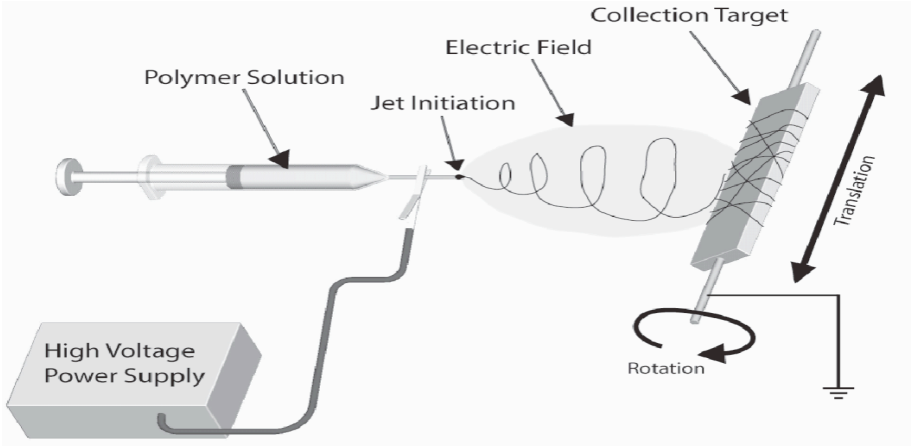


Fig. 7. Schematic of the Electrospinning process [16].

www.manaraa.com

Materials and Methods

Electrospinning

PDO (Ethicon Inc.) and soluble elastin (Elastin Products Co., Inc) were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (TCI America) in concentrations of 100 mg/ml and 200 mg/ml respectively. These solutions were blended in the ratios of 50:50, 70:30 and 90:10 (PDO:elastin) by volume. Pure PDO (100:0) and pure elastin (0:100) were also used in the study. In addition PDO was also dissolved in the concentrations of 60, 80, 100, 120 and 140 mg/ml. These solutions were then loaded into a Becton Dickinson syringe (5.0 ml) with an 18 gauge blunt tip needle and placed in a KD scientific syringe pump to be dispensed at a rate of 4-6 ml/hr (higher flow rates were used for high PDO ratio blends). Solutions were then electrospun onto a flat rotating stainless steel mandrel (2.5 cm wide x 10.2 cm long x 0.3 cm thick) to produce a flat sheet. All electrospinning was performed at an applied voltage of 22kV, while the mandrel was rotated at a rate of 500 rpm and placed 12 cm away from the needle tip [5].

Cross Linking

Soluble elastin being a natural polymer lacks substantial strength upon hydration. Therefore, all elastin rich electrospun scaffolds were crosslinked using a method developed by Barnes et al. [35] . Briefly, the scaffolds were soaked for 18 hours in 167 mM (50 fold

www.manaraa.com

molar excess for 200 mg/ml of elastin) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Sigma Aldrich) solution in ethanol, followed by a 2 hour rinse in 0.1 M sodium phosphate. PDO and 90:10 blend did not require crosslinking, but were processed the same way in order to exclude any variability in the results.

Permeability Measurement

Circular discs were punched out of the scaffolds using a 10 mm biopsy punch and the permeability was individually measured using the simple flowmeter described in [36]. Briefly, the amount of time for a known volume of water to flow through a bound area of the scaffold was recorded and calculations were done using equation 1.

$$\text{Permeability} = \frac{Q}{Aht} \qquad (1)$$

Where Q is the volume of water passed through the scaffold (ml) in time t (min) and A and h are the area (cm^2) and the thickness (cm) of the scaffold respectively. The distance between the water source (horizontal 10 mL pipette) and the scaffold was maintained at a level to produce a pressure head of 120 mm Hg (Fig.8).

www.manaraa.com

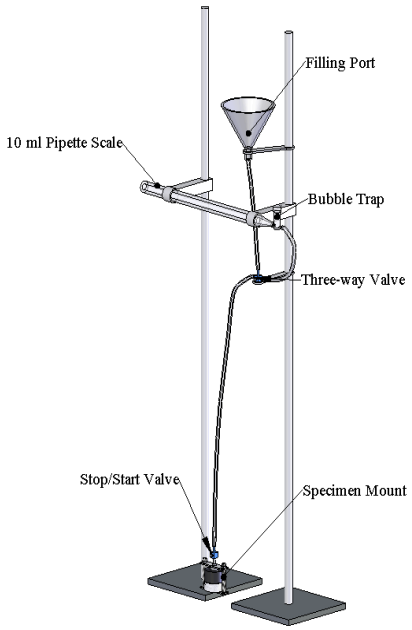


Fig. 8. Diagram of the permeability measurement device used in the study [36].

Cell Culture

Human peripheral blood monocytes (CRL-9855, ATCC) were seeded into culture at a density of $6x10^7$ cells per 75 cm^2 flask in Dulbecco's modified eagle medium (high glucose) with 20% heat inactivated fetal bovine serum and 10% heat inactivated AB+ serum. This cell concentration provided a high seeding density for differentiation of monocytes to macrophages. Macrophages were obtained after 21 days of culture. It has been documented that serum proteins found in Human AB+ Serum (e.g. Human Immunoglobulin (IgG)) promote maturation by interacting with monocytes via Fc receptors [37]. Monocytes also show macrophage characteristics such as increased

www.manaraa.com

cytolysis and intracellular levels of lysosomal enzymes in the presence of autologous serum [38]. Godiska et al. reported that the chemoattractant macrophage derived chemokine (MDC) has a very specific pattern of expression and is highly expressed by macrophages and monocyte-derived dendritic cells, but is not expressed by freshly isolated monocytes, granulocytic cells, or natural killer cells [39]. Macrophages were then adapted for culture in Macrophage-SFM (serum free media) (Invitrogen). A sequential adaptation technique was used to adapt the cells as a drastic change from 30% serum to a serum free media would have been too stressful for the cells. The ratio of serum supplemented media (SSM) to serum free media (SFM) was gradually reduced every week. For the first week cells were cultured in 80:20 (SSM:SFM). In the following week the ratio of media used was 50:50 and then a gradual increase in the amount of SFM media was made as the cells were cultured in 20:80, followed by 0:100. No changes in morphology or viability were observed after the adaptation. Monocytes and macrophages were cultured at a density of 50,000 cells/well in a 12 well plate and the supernatants were collected and stored at day 7, 14, 21 and 28 until needed for the MDC expression analysis.

Cell Seeding on Scaffolds

Macrophages were adapted for culture in serum free media (Invitrogen). A serum free medium formulation was chosen to exclude the contribution of serum derived angiogenesis factors in the study. Conventional tissue culture techniques that frequently

www.manaraa.com

employ serum stimulate cells by various biological modifiers such as hormones, proteins and other biomolecules.

Prior to cell seeding the scaffolds were disinfected by soaking in ethanol for 10 min followed by repeated rinses in phosphate buffered saline (PBS). The macrophages were then seeded at a density of 400,000 cells/well on electrospun (10 mm) disks of PDO (60 to 140 mg/ml), elastin, PDO:elastin blends (50:50, 70:30 and 90:10) and on tissue culture plastic (TCP) in a 48 well plate. The supernatants were collected on day 7, 14, 21 and 28 and stored at -70°C until needed. One disc of each ratio was also removed at the above mentioned time points, fixed in 10% formalin and processed for histology (Hematoxylin and Eosin staining; H&E).

Enzyme Linked Immunosorbent Assay (ELISA)

The growth factor expression was quantified using Enzyme Linked Immunosorbent Assay (ELISA). The cell culture supernatants were thawed and each ELISA for specific growth factors (VEGF, aFGF, bFGF, TGF-β1) to be evaluated was carried out using construction kits (Antigenix America) as per manufacturer's instructions. The standard curve was generated using log-log fit (Soft MaxPro 4.8) and its linear part was used to determine the concentration of the factor in the supernatant. Latent TGF-β1 was activated to its immuno-reactive form using the procedure outlined in the kit's instructions.

www.manaraa.com

Statistical Analysis

All statistical analysis of the data was based on a Kruskal-Wallis one way analysis of variance on ranks and a Tukey-Kramer pairwise multiple comparison procedure (α=0.05) performed with JMP® IN 7 statistical software (SAS Institute).

www.manaraa.com

Results and Discussion

Permeability Measurement

Statistically, PDO was significantly more permeable than the rest of the materials and 90:10 was more permeable than 70:30 and 50:50 (Fig.9). Therefore, cell infiltration can be directly related to the permeability of the scaffold. It can be concluded that PDO and the 90:10 blend have high porosities as pore size is directly proportional to the permeability and the fiber diameter of the electrospun scaffolds. Sanders et al. reported reduced macrophage density on small diameter fibers (<6 μm) attributed to a reduced cell material contact surface area [40]. The permeability test results for the different concentrations of the PDO scaffold showed that 100 mg/ml and 120 mg/ml scaffolds were statistically significantly different from the 60 mg/ml and 80 mg/ml scaffolds. Also, the permeability within each of these two groups was not significantly different from one another. However, the 140 mg/ml scaffold was not statistically different from the 60 mg/ml and 80 mg/ml scaffolds.

It has been documented that pore size of PDO scaffolds increases linearly with concentration. The reason why the permeability peaks at about 120 mg/ml and falls dramatically for 140 mg/ml scaffold could be attributed to the pore collapse of these structures. It could have been that the pores and fibers were so large that the pores actually collapsed under the water pressure and the void spaces were filled with fibers since the entire scaffold is dynamic and can move around on a micron scale level (Fig.10).

www.manaraa.com

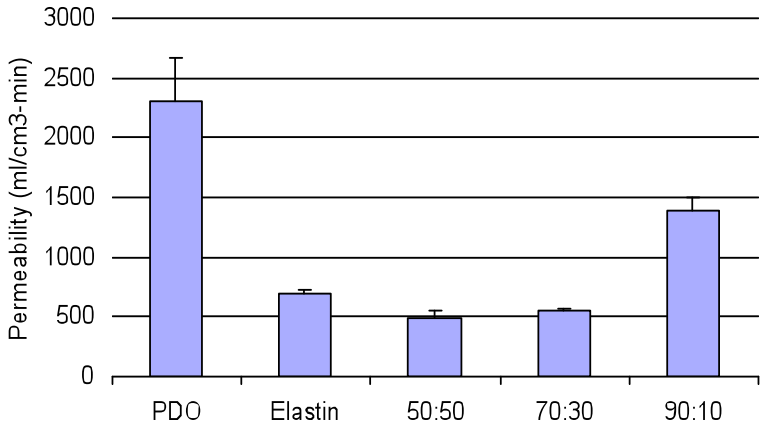


Fig. 9. Permeability measurements for samples of electrospun PDO:elastin scaffolds. Error bars indicate standard error of the mean.

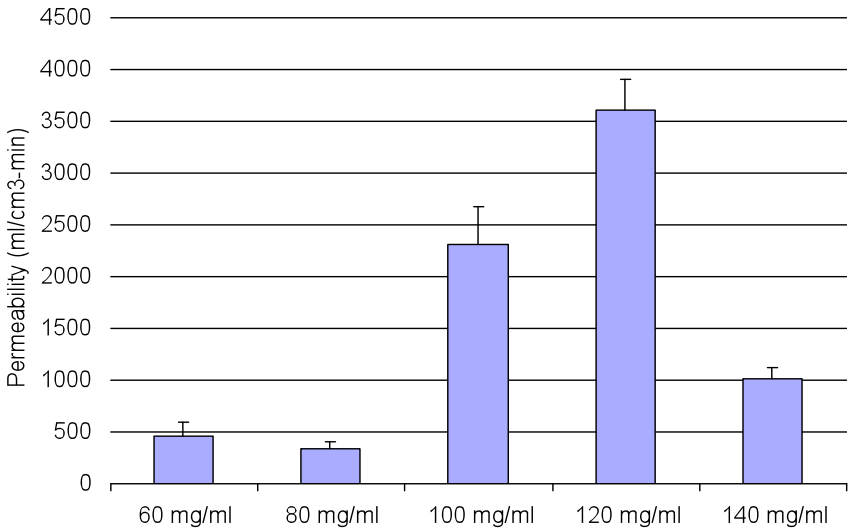


Fig. 10. Permeability measurements for samples of electrospun PDO scaffolds of varying concentration. Error bars indicate standard error of the mean.

www.manaraa.com

Macrophage Derived Chemokine ELISA

In order to confirm the maturation of monocytes to macrophages a Macrophage Derived Chemokine (MDC) (R&D Systems) ELISA was performed. This was based on the fact that macrophages produce MDC constitutively while monocytes release it only upon stimulation [39]. Significant statistical differences were found on day 21 of culture (Fig.11). Statistical differences were not observed on the other days. However, it was seen that both monocytes and macrophages were producing comparable amounts of MDC. This can be explained by taking into account the fact that a mixed cell population was purchased from ATCC. In addition, it has been documented that after 7 days of culture in serum free media monocytes transform into macrophages [1]. In another example, human peripheral blood monocytes cultured in 10% autologous serum were shown to have differentiated to macrophages after 5-6 days [41, 42]. They also reported that the number of adherent cells were similar when grown in the presence of AB serum or autologous serum [42]. The MDC expression was also found to be consistent from day 7 to 28, however it is unclear what the baseline measurements (day 0) would reveal.

www.manaraa.com

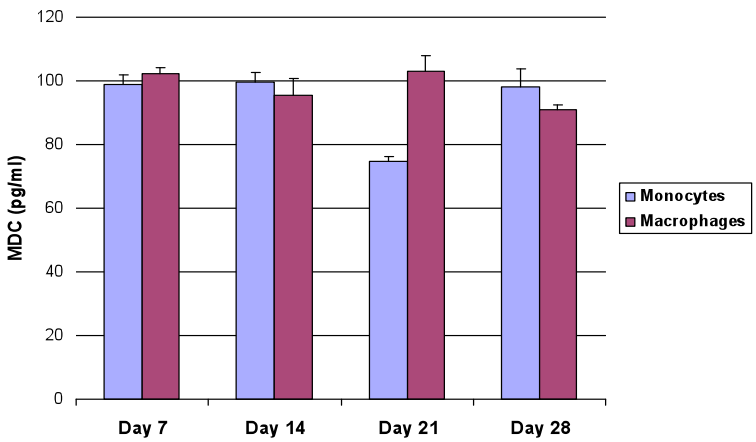


Fig. 11. Quantification of MDC production by macrophages and monocytes. Error bars indicate standard error of the mean.

Macrophage Factor Secretion (Quantification by ELISA)

The growth factor expression analysis revealed that macrophages released high amounts of VEGF and aFGF with a steady increase from day 7 to 28. The expression of TGF-β1 was relatively low. The production of bFGF was below the detectable level (10 pg/ml) of the kit. The activation of macrophages in this case is theorized to be integrin mediated. Integrins are a large family of cell surface receptors that mediate cell-extracellular matrix and intracellular interactions. Enough information is not available to provide insight into how activation may occur when cells encounter engineered materials composed of proteins and synthetic polymers.

As shown in figure 12, the expression of VEGF on biomaterial adherent macrophages did not vary considerably among the different scaffolds. The secretion of aFGF and VEGF was found to remain largely independent of the scaffold material. The production of both aFGF and VEGF showed a steady increase from day 7 to 28. VEGF

www.manaraa.com

secretion on day 28 was found to be statistically higher than day 7 on all materials. The secretion of aFGF on day 28 was found to be higher than both day 7 and 14 on all materials. This indicates that the production of both aFGF and VEGF was found to be significantly higher in later stages of cell culture.

The expression of TGF-β1 gradually decreased from day 7 to 21 and came back up again on day 28. The only exceptions to this trend were the PDO and the 70:30 scaffold. Statistically significant differences were found in TGF-β1 secretion between PDO and PDO:elastin (90:10) blend on day 7. Macrophages secrete TGF-β1 as well as its binding protein. The binding protein inhibits the interaction of TGF-β1 with its cell surface receptor, thus showing that TGF-β1-binding protein complex is biologically inactive. This interaction modulates the expression and action of TGF-β1.

Figure 13 shows the growth factor expression on PDO scaffolds of varying concentrations. It was observed that the growth factor secretion was significantly higher on high PDO concentration scaffolds. The only exception to this trend was the secretion of TGF- β1 and aFGF on the 80 mg/ml scaffold which was significantly higher than the 140 mg/ml scaffold on day 21. Similar results were obtained again in the expression of TGF-β1 as its concentration went progressively low from day 7 to 21 and came back up again on day 28. The only exception to this trend was the 100 mg/ml PDO scaffold. 60 mg/ml scaffold showed a rather constant level of TGF-β1 production from day 7 to 28. VEGF secretion was found to be largely material independent. It was also found that VEGF production was significantly higher on day 28 as compared to day 7 on all concentrations of PDO. A detailed statistical report is provided in the appendix for other statistical differences not reported in the text.

www.manaraa.com

It was observed that the growth factor secretion on scaffolds was comparable to the growth factor secretion on tissue culture plastic. This shows that the materials are neither elevating nor suppressing the secretory function of macrophages. It can be anticipated that these materials as vascular implants will support natural tissue regeneration process with minimum risk of tumors or undesirable inflammatory reactions. Too much of growth factor secretion can lead to uncontrolled cell migration and proliferation. There are reports of 'leaky' blood vessels which exhibit fibrosis, oedema, inflammation and haemorrhagic ulcers due to over expression of VEGF and TGF-β1 [15, 43].

The degradation activity of macrophages was observed to be higher on scaffolds containing high ratios of PDO. This can be attributed to the high permeability of these materials, which enabled good infiltration of macrophages throughout the entire thickness of the scaffold. Permeability is directly related to the pore size and the fiber diameter. It is known that increase in fiber size is associated with an increase in pore size. Drastic pH changes were observed in the cell culture media on various samples. Low pH was generated in the microenvironment of the scaffolds containing high PDO ratios, indicating that they are undergoing degradation. Previous studies have shown that low pH stimulates macrophages to release growth factors [44].

www.manaraa.com

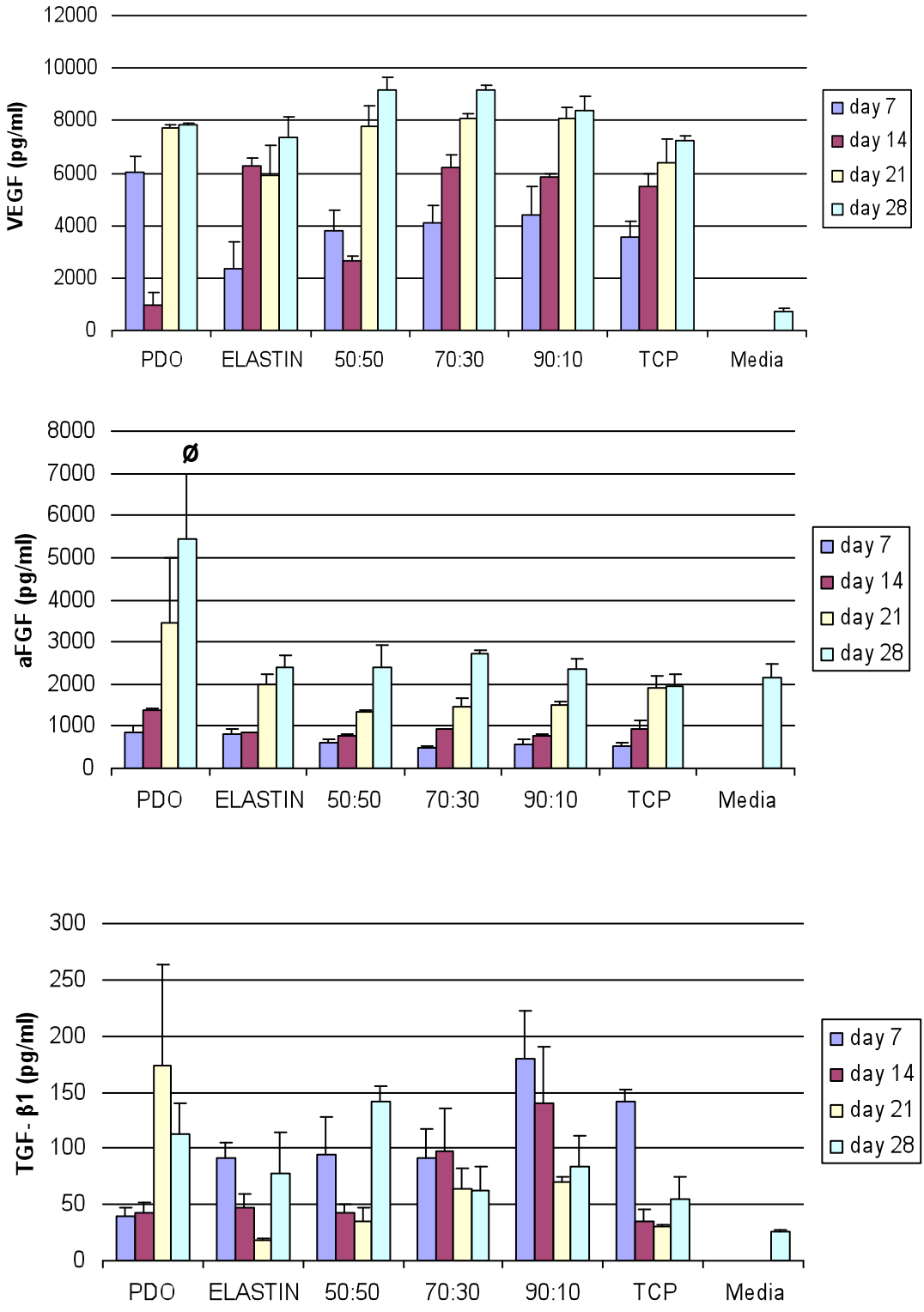


Fig. 12. Quantification of macrophage secretory activity on PDO:elastin scaffolds using ELISA. Error bars indicate standard error of the mean. Symbols '*' and 'ø' indicate a statistically significant difference from the control groups, media and TCP respectively ($p<0.05$).

www.manaraa.com

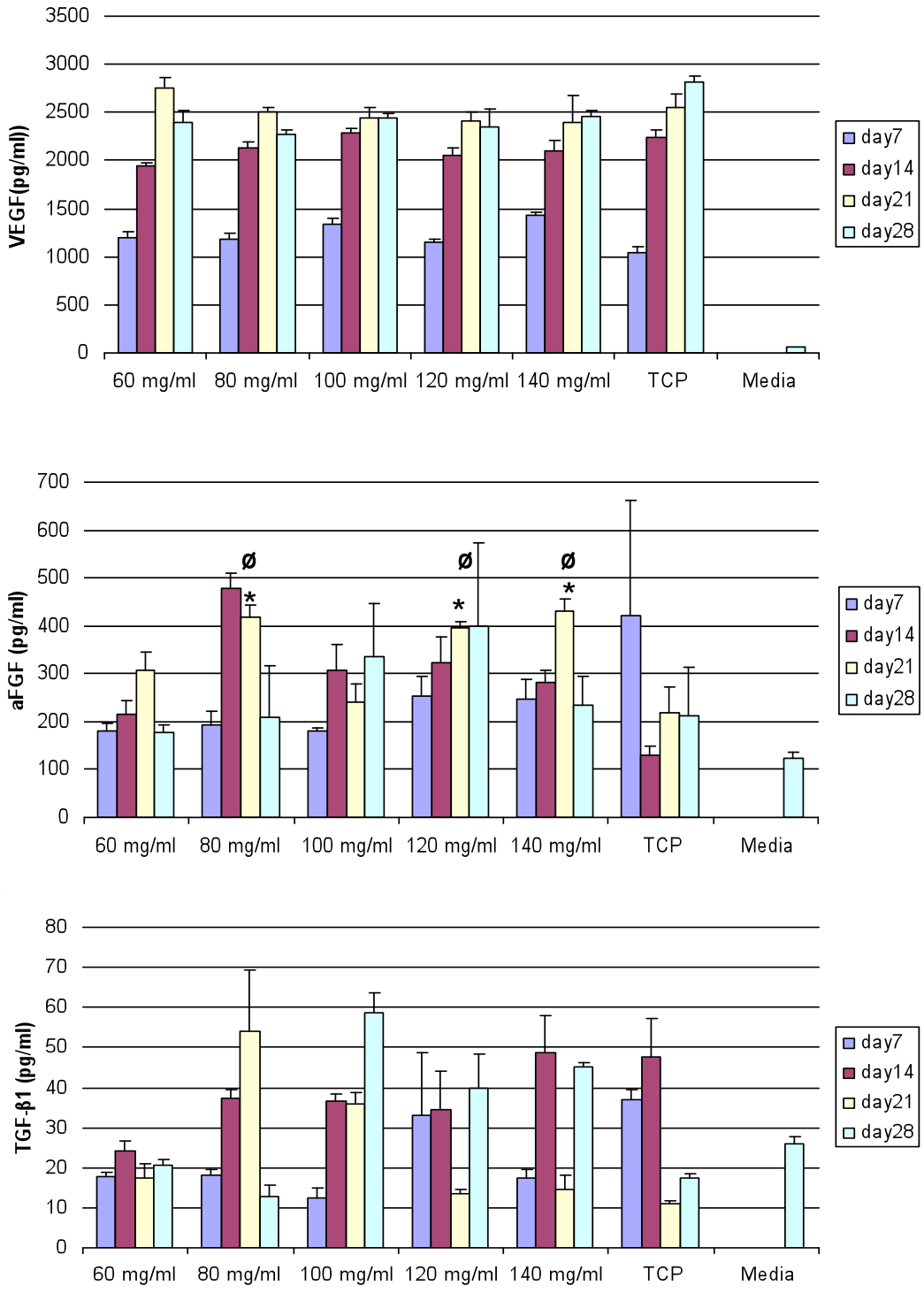


Fig. 13. Quantification of macrophage secretory activity on PDO scaffolds of varying concentrations using ELISA. Error bars indicate standard error of the mean. Symbols '*' and 'ø' indicate a statistically significant difference from the control groups, media and TCP respectively ($p<0.05$).

www.manaraa.com

Histology

Histology revealed greater cell infiltration into the fibrous networks of the PDO and 90:10 scaffolds (Fig.14). The cells migrated to some extent on the elastin scaffold and remained on the surface of the 50:50 and the 70:30 blend. It has been documented that when angiogenesis occurs in a remodeling situation such as a bioresorbable vascular prosthetic, it is accompanied by an inflammatory infiltration composed of monocytes and macrophages. The abolishment of this infiltration dramatically reduces the angiogenesis and wound healing response [45]. Also, it has been reported that the number of macrophages directly correlate to the degree of angiogenesis [46, 47]. To evaluate the effect of porosity on the infiltration of macrophages in the scaffold, PDO scaffolds were electrospun in the range of 60 mg/ml to 140 mg/ml. The increasing concentration correlates to the increasing fiber diameter and pore size. It has been documented that the diameter of PDO fibers increased linearly from 0.18 to 1.4μm range as the solution concentration increased in the range 42-167 mg/ml. The pore size however exhibited a non-linear relation with solution concentration. It was found to increase from 0.5 μm^2 to 24.5 μm^2 as solution concentration increased from 42 to 167 mg/ml. The statistical analysis indicated that the 42–56 mg/ml pore areas were significantly different from the 71–167 mg/ml pore areas, though the pore areas within each of those groups were not significantly different from each other. Porosity and surface area are two key variables influencing interaction of structures with the host environment. For example, a highly porous structure with a large surface area may be more favorable to cell attachment and infiltration of cellular components [16]. The current study is in agreement with this fact.

www.manaraa.com

As shown in Figure 15, the macrophages did not migrate at all into the fibrous networks of 60 mg/ml and 80 mg/ml PDO scaffolds. They largely remained on the surface and did not migrate through even at day 21 of culture. On the other hand, scaffolds spun at concentrations of 100 mg/ml, 120 mg/ml and 140 mg/ml showed increased cell migration and infiltration throughout the entire thickness of the scaffold. Thus, it can be concluded that the macrophages did not infiltrate elastin and 50:50 scaffolds due to the highly compact and dense structure of these scaffolds which led to low pore size.

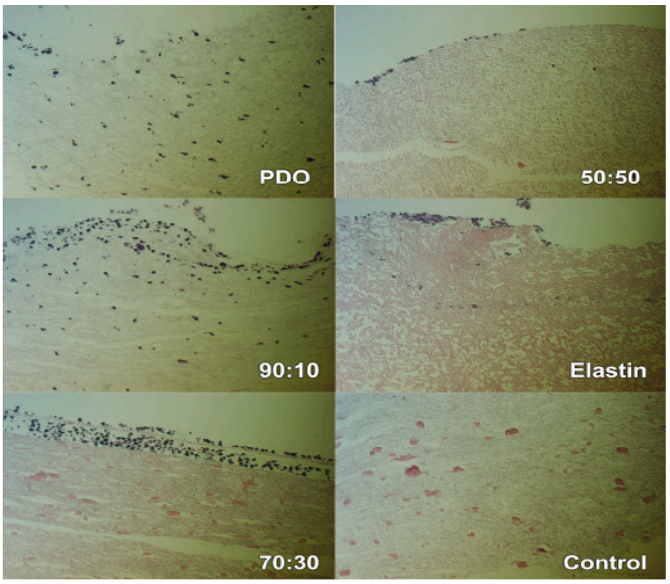


Fig. 14. Histology (H&E) performed at day 21(20x magnification).

www.manaraa.com

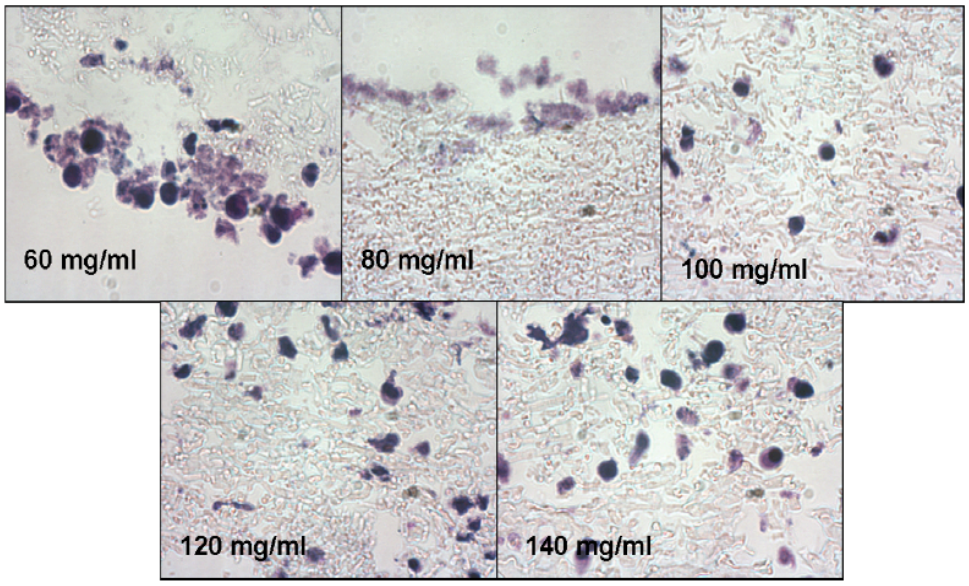


Fig. 15. Histology performed on various concentrations of PDO at day 21 (40x magnification).

www.manaraa.com

Conclusion

Biomaterials upon implantation acquire a layer of host proteins prior to interacting with host cells. The type, concentration and conformations of these surface adsorbed proteins are dependent on the material surface properties [14]. Macrophage adhesion to the material surface adherent protein layer is integrin mediated. It provides intracellular signals that dictate macrophage behavior. A cascade of events are triggered that affect cytoskeletal rearrangements and formation of adhesion structures. Macrophages undergo cytoskeletal remodeling to spread over the material surface. When cells are properly adhered to the cell surface, integrin signaling mediates survival. Disruption of the adhesion signals lead to anoikis (term for apoptosis induced by cell detachment from its supportive matrix) [14]. The adherent macrophages become activated in order to phagocytose the biomaterial. This is followed by cytokine secretion that directs the inflammatory and wound healing response to the biomaterial. *In vitro* testing of growth factor profiles released by adherent macrophages can be an initial means of assaying biocompatibility [14].

The study provides an angiogenic assessment of macrophage implant interaction by quantifying the growth factor secretion in the cell culture supernatants. It was observed that the growth factor secretion profiles for VEGF and aFGF attained similar levels around day 21 and 28 for all materials. Previous studies have shown that macrophages do not produce bFGF on biomaterials unless they are stimulated by

www.manaraa.com

concanavalin A or lipopolysaccharide [1]. The fact that bFGF expression is strong in chronic inflammations and weak in healthy tissue is also known. It has also been established in that bFGF is not absolutely mandatory for angiogenesis and that vascularization does occur in its absence [12].

Elastin incorporation into the scaffolds was done to improve the *in vitro* bioactivity of the matrix and also to improve the mechanical properties of the material [5]. The study however shows better results on scaffolds with high PDO ratios as compared to the elastin rich scaffolds. The increased cell migration and proliferation on PDO and the 90:10 blend can be largely attributed to the high porosities of these materials. Therefore, it can be concluded that scaffolds with high PDO ratios were more conducive to tissue regeneration and angiogenesis with minimum risk of thrombosis.

www.manaraa.com

Literature Cited

www.manaraa.com

References

1. Dagtekin, G., et al., *Modulation of angiogenic functions in human macrophages by biomaterials.* Biomaterials, 2003. **24**(20): p. 3395-401.
2. Xia, Z. and J.T. Triffitt, *A review on macrophage responses to biomaterials.* Biomed Mater, 2006. **1**(1): p. R1-9.
3. Zakrzewska, M., E. Marcinkowska, and A. Wiedlocha, *FGF-1: from biology through engineering to potential medical applications.* Crit Rev Clin Lab Sci, 2008. **45**(1): p. 91-135.
4. Huang L, M.R.A., Apkarian R P, Pourdeyhimi B, Conticello V P, Chaikof E L, *Generation of synthetic elastin-mimetic small diameter fibers and fibre networks.* Macromolecules, 2000. **33**: p. 2989-97.
5. Sell, S.A., et al., *Electrospun polydioxanone-elastin blends: potential for bioresorbable vascular grafts.* Biomed Mater, 2006. **1**(2): p. 72-80.
6. Patel, A., et al., *Elastin biosynthesis: The missing link in tissue-engineered blood vessels.* Cardiovasc Res, 2006. **71**(1): p. 40-9.
7. Sunderkotter, C., et al., *Macrophages and angiogenesis.* J Leukoc Biol, 1994. **55**(3): p. 410-22.
8. Lewis, C.E. and J.O.D. McGee, *The macrophage.* 1992.
9. Janeway, *Immunobiology.* 2005.
10. Lamagna, C., M. Aurrand-Lions, and B.A. Imhof, *Dual role of macrophages in tumor growth and angiogenesis.* J Leukoc Biol, 2006. **80**(4): p. 705-13.

www.manaraa.com

11. Gordon, S., *Alternative activation of macrophages.* Nat Rev Immunol, 2003. **3**(1): p. 23-35.

12. Shiu, Y.T., et al., *The role of mechanical stresses in angiogenesis.* Crit Rev Biomed Eng, 2005. **33**(5): p. 431-510.

13. Greisler, H.P., et al., *Derivation of neointima in vascular grafts.* Circulation, 1988. **78**(3 Pt 2): p. I6-12.

14. Anderson, J.M., A. Rodriguez, and D.T. Chang, *Foreign body reaction to biomaterials.* Semin Immunol, 2008. **20**(2): p. 86-100.

15. Sunderkotter, C., et al., *Macrophage-derived angiogenesis factors.* Pharmacol Ther, 1991. **51**(2): p. 195-216.

16. Boland, E.D., et al., *Electrospinning polydioxanone for biomedical applications.* Acta Biomater, 2005. **1**(1): p. 115-23.

17. Swartbol, P., et al., *Tumor necrosis factor-alpha and interleukin-6 release from white blood cells induced by different graft materials in vitro are affected by pentoxifylline and iloprost.* J Biomed Mater Res, 1997. **36**(3): p. 400-6.

18. Wesolowski, S.A., et al., *The compound prosthetic vascular graft: a pathologic survey.* Surgery, 1963. **53**: p. 19-44.

19. Ruderman, R.J., et al., *A partially biodegradable vascular prosthesis.* Trans Am Soc Artif Intern Organs, 1972. **18**(0): p. 30-7.

20. Bowald, S., C. Busch, and I. Eriksson, *Arterial regeneration following polyglactin 910 suture mesh grafting.* Surgery, 1979. **86**(5): p. 722-9.

www.manaraa.com

21. Greisler, H.P., *Arterial regeneration over absorbable prostheses.* Arch Surg, 1982. **117**(11): p. 1425-31.

22. Greisler, H.P., et al., *Arterial regenerative activity after prosthetic implantation.* Arch Surg, 1985. **120**(3): p. 315-23.

23. Atala, A. and R.P. Lanza, *Methods of Tissue Engineering.* 2002.

24. Greisler, H.P., et al., *Compound polyglactin 910/polypropylene small vessel prostheses.* J Vasc Surg, 1987. **5**(4): p. 572-83.

25. Greisler, H.P., et al., *Polyglactin 910/polydioxanone bicomponent totally resorbable vascular prostheses.* J Vasc Surg, 1988. **7**(5): p. 697-705.

26. Greisler, H.P., et al., *Arterial regeneration over polydioxanone prostheses in the rabbit.* Arch Surg, 1987. **122**(6): p. 715-21.

27. Schwarcz, T.H., et al., *Prostaglandin content of tissue lining vascular prostheses.* Curr Surg, 1987. **44**(1): p. 18-21.

28. Zhang, Y., et al., *Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds.* J Biomed Mater Res B Appl Biomater, 2005. **72**(1): p. 156-65.

29. Casper, C.L., et al., *Functionalizing electrospun fibers with biologically relevant macromolecules.* Biomacromolecules, 2005. **6**(4): p. 1998-2007.

30. Boland, E.D., et al., *Electrospinning collagen and elastin: preliminary vascular tissue engineering.* Front Biosci, 2004. **9**: p. 1422-32.

31. Brodbeck, W.G., et al., *Biomaterial surface chemistry dictates adherent monocyte/macrophage cytokine expression in vitro.* Cytokine, 2002. **18**(6): p. 311-9.

www.manaraa.com

32. Salthouse, T.N., *Some aspects of macrophage behavior at the implant interface.* Journal of Biomedical Materials Research, 1984. **18**: p. 395–401.

33. Ratner, B.D. and S.J. Bryant, *Biomaterials: where we have been and where we are going.* Annu Rev Biomed Eng, 2004. **6**: p. 41-75.

34. Zheng-Ming Huang, Y.-Z.Z., M. Kotaki, S. Ramakrishna, *A review on polymer nanofibers by electrospinning and their applications in nanocomposites.* Composite Science and Technology, 2003(63): p. 2223-2253.

35. Barnes, C.P., et al., *Cross-linking electrospun type II collagen tissue engineering scaffolds with carbodiimide in ethanol.* Tissue Eng, 2007. **13**(7): p. 1593-605.

36. Sell, S., et al., *Scaffold permeability as a means to determine fiber diameter and pore size of electrospun fibrinogen.* J Biomed Mater Res A, 2008. **85**(1): p. 115-26.

37. Akiyama, Y., et al., *Effects of adherence, activation and distinct serum proteins on the in vitro human monocyte maturation process.* J Leukoc Biol, 1988. **43**(3): p. 224-31.

38. Musson, R.A., *Human serum induces maturation of human monocytes in vitro. Changes in cytolytic activity, intracellular lysosomal enzymes, and nonspecific esterase activity.* Am J Pathol, 1983. **111**(3): p. 331-40.

39. Godiska, R., et al., *Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells.* J Exp Med, 1997. **185**(9): p. 1595-604.

www.manaraa.com

40. Sanders, J.E., C.E. Stiles, and C.L. Hayes, *Tissue response to single-polymer fibers of varying diameters: evaluation of fibrous encapsulation and macrophage density.* J Biomed Mater Res, 2000. **52**(1): p. 231-7.

41. Stafforini, D.M., et al., *Human macrophages secret platelet-activating factor acetylhydrolase.* J Biol Chem, 1990. **265**(17): p. 9682-7.

42. Seager Danciger, J., et al., *Method for large scale isolation, culture and cryopreservation of human monocytes suitable for chemotaxis, cellular adhesion assays, macrophage and dendritic cell differentiation.* J Immunol Methods, 2004. **288**(1-2): p. 123-34.

43. Nillesen, S.T., et al., *Increased angiogenesis and blood vessel maturation in acellular collagen-heparin scaffolds containing both FGF2 and VEGF.* Biomaterials, 2007. **28**(6): p. 1123-31.

44. Galletti, P.M., et al., *Experience with fully bioresorbable aortic grafts in the dog.* Surgery, 1988. **103**(2): p. 231-41.

45. Moldovan, N.I., *Role of monocytes and macrophages in adult angiogenesis: a light at the tunnel's end.* J Hematother Stem Cell Res, 2002. **11**(2): p. 179-94.

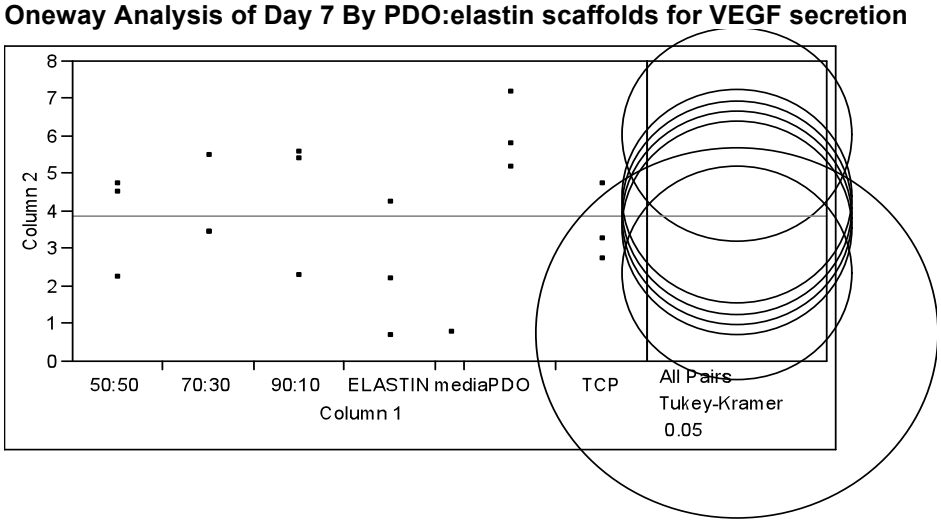
46. Torisu, H., et al., *Macrophage infiltration correlates with tumor stage and angiogenesis in human malignant melanoma: possible involvement of TNFalpha and IL-1alpha.* Int J Cancer, 2000. **85**(2): p. 182-8.

47. Ono, M., et al., *Biological implications of macrophage infiltration in human tumor angiogenesis.* Cancer Chemother Pharmacol, 1999. **43 Suppl**: p. S69-71.

www.manaraa.com

APPENDIX A: Statistical Analysis

Oneway Analysis of Day 7 By PDO:elastin scaffolds for VEGF secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	PDO	90:10	70:30	50:50	TCP	ELASTIN	media
PDO	-4.0523	-2.4293	-2.1203	-1.8493	-1.5883	-0.3780	-0.4572
90:10	-2.4293	-4.0523	-3.7433	-3.4723	-3.2113	-2.0010	-2.0802
70:30	-2.1203	-3.7433	-4.0523	-3.7813	-3.5203	-2.3100	-2.3892
50:50	-1.8493	-3.4723	-3.7813	-4.0523	-3.7913	-2.5810	-2.6602
TCP	-1.5883	-3.2113	-3.5203	-3.7913	-4.0523	-2.8420	-2.9212
ELASTIN	-0.3780	-2.0010	-2.3100	-2.5810	-2.8420	-4.0523	-4.1316
media	-0.4572	-2.0802	-2.3892	-2.6602	-2.9212	-4.1316	-7.0189

Positive values show pairs of means that are significantly different.

Level		Mean
PDO	A	6.0166667
90:10	A	4.3936667
70:30	A	4.0846667
50:50	A	3.8136667
TCP	A	3.5526667
ELASTIN	A	2.3423333
media	A	0.7430000

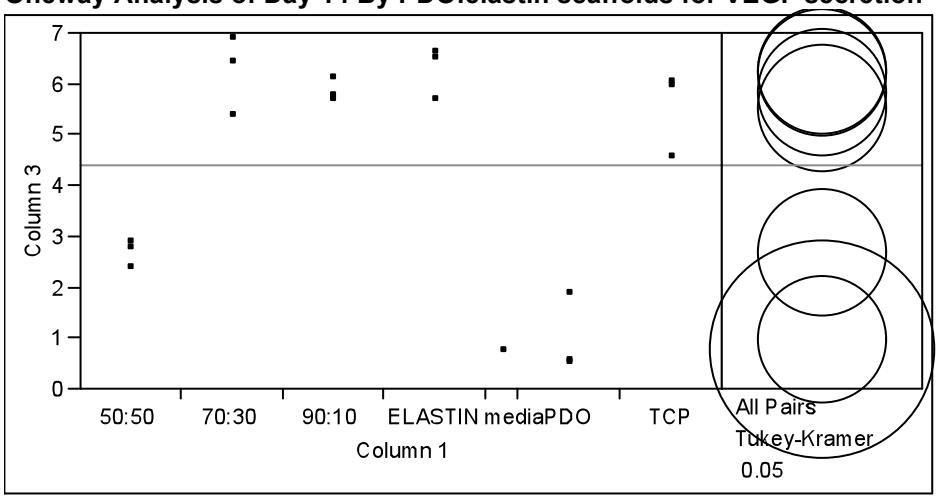
Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference

www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
PDO	media	5.273667	-0.45722	11.00455	
PDO	ELASTIN	3.674333	-0.37801	7.72668	
90:10	media	3.650667	-2.08022	9.38155	
70:30	media	3.341667	-2.38922	9.07255	
50:50	media	3.070667	-2.66022	8.80155	
TCP	media	2.809667	-2.92122	8.54055	
PDO	TCP	2.464000	-1.58835	6.51635	
PDO	50:50	2.203000	-1.84935	6.25535	
90:10	ELASTIN	2.051333	-2.00101	6.10368	
PDO	70:30	1.932000	-2.12035	5.98435	
70:30	ELASTIN	1.742333	-2.31001	5.79468	
PDO	90:10	1.623000	-2.42935	5.67535	
ELASTIN	media	1.599333	-4.13155	7.33022	
50:50	ELASTIN	1.471333	-2.58101	5.52368	
TCP	ELASTIN	1.210333	-2.84201	5.26268	
90:10	TCP	0.841000	-3.21135	4.89335	
90:10	50:50	0.580000	-3.47235	4.63235	
70:30	TCP	0.532000	-3.52035	4.58435	
90:10	70:30	0.309000	-3.74335	4.36135	
70:30	50:50	0.271000	-3.78135	4.32335	
50:50	TCP	0.261000	-3.79135	4.31335	

Oneway Analysis of Day 14 By PDO:elastin scaffolds for VEGF secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	ELASTIN	70:30	90:10	TCP	50:50	PDO	media
ELASTIN	-1.7627	-1.7277	-1.3520	-1.0160	1.8230	3.5176	3.0188
70:30	-1.7277	-1.7627	-1.3870	-1.0510	1.7880	3.4826	2.9838
90:10	-1.3520	-1.3870	-1.7627	-1.4267	1.4123	3.1070	2.6081
TCP	-1.0160	-1.0510	-1.4267	-1.7627	1.0763	2.7710	2.2721
50:50	1.8230	1.7880	1.4123	1.0763	-1.7627	-0.0680	-0.5669
PDO	3.5176	3.4826	3.1070	2.7710	-0.0680	-1.7627	-2.2615
media	3.0188	2.9838	2.6081	2.2721	-0.5669	-2.2615	-3.0531

Positive values show pairs of means that are significantly different.

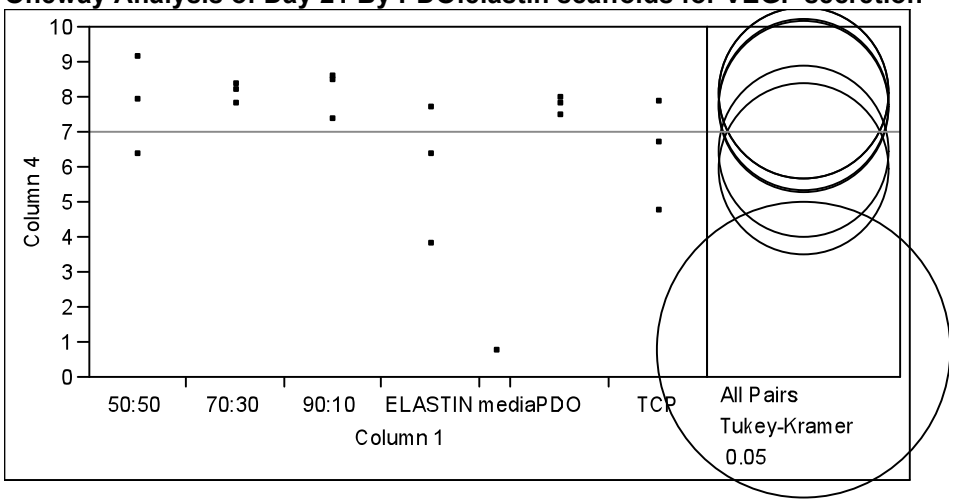
www.manaraa.com

Level			Mean
ELASTIN	A		6.2546667
70:30	A		6.2196667
90:10	A		5.8440000
TCP	A		5.5080000
50:50		B	2.6690000
PDO		B	0.9743333
media		B	0.7430000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
ELASTIN	media	5.511667	3.01882	8.004518	
70:30	media	5.476667	2.98382	7.969518	
ELASTIN	PDO	5.280333	3.51762	7.043045	
70:30	PDO	5.245333	3.48262	7.008045	
90:10	media	5.101000	2.60815	7.593851	
90:10	PDO	4.869667	3.10695	6.632379	
TCP	media	4.765000	2.27215	7.257851	
TCP	PDO	4.533667	2.77095	6.296379	
ELASTIN	50:50	3.585667	1.82295	5.348379	
70:30	50:50	3.550667	1.78795	5.313379	
90:10	50:50	3.175000	1.41229	4.937712	
TCP	50:50	2.839000	1.07629	4.601712	
50:50	media	1.926000	-0.56685	4.418851	
50:50	PDO	1.694667	-0.06805	3.457379	
ELASTIN	TCP	0.746667	-1.01605	2.509379	
70:30	TCP	0.711667	-1.05105	2.474379	
ELASTIN	90:10	0.410667	-1.35205	2.173379	
70:30	90:10	0.375667	-1.38705	2.138379	
90:10	TCP	0.336000	-1.42671	2.098712	
PDO	media	0.231333	-2.26152	2.724185	
ELASTIN	70:30	0.035000	-1.72771	1.797712	

Oneway Analysis of Day 21 By PDO:elastin scaffolds for VEGF secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

www.manaraa.com

Abs(Dif)-LSD	90:10	70:30	50:50	PDO	TCP	ELASTIN	media
90:10	-3.4694	-3.4548	-3.1484	-3.0844	-1.7768	-1.2854	2.4512
70:30	-3.4548	-3.4694	-3.1631	-3.0991	-1.7914	-1.3001	2.4365
50:50	-3.1484	-3.1631	-3.4694	-3.4054	-2.0978	-1.6064	2.1302
PDO	-3.0844	-3.0991	-3.4054	-3.4694	-2.1618	-1.6704	2.0662
TCP	-1.7768	-1.7914	-2.0978	-2.1618	-3.4694	-2.9781	0.7585
ELASTIN	-1.2854	-1.3001	-1.6064	-1.6704	-2.9781	-3.4694	0.2672
media	2.4512	2.4365	2.1302	2.0662	0.7585	0.2672	-6.0092

Positive values show pairs of means that are significantly different.

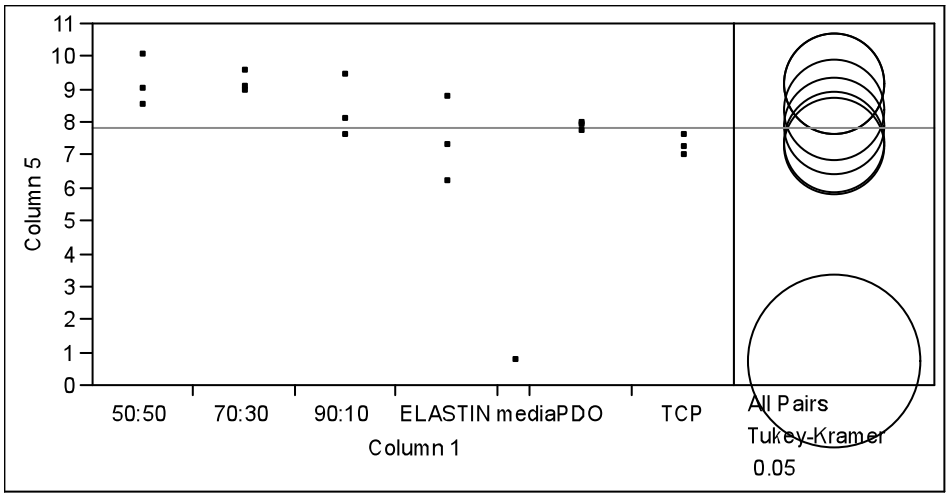
Level		Mean
90:10	A	8.1006667
70:30	A	8.0860000
50:50	A	7.7796667
PDO	A	7.7156667
TCP	A	6.4080000
ELASTIN	A	5.9166667
media	B	0.7430000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
90:10	media	7.357667	2.45115	12.26418	
70:30	media	7.343000	2.43649	12.24951	
50:50	media	7.036667	2.13015	11.94318	
PDO	media	6.972667	2.06615	11.87918	
TCP	media	5.665000	0.75849	10.57151	
ELASTIN	media	5.173667	0.26715	10.08018	
90:10	ELASTIN	2.184000	-1.28543	5.65343	
70:30	ELASTIN	2.169333	-1.30010	5.63876	
50:50	ELASTIN	1.863000	-1.60643	5.33243	
PDO	ELASTIN	1.799000	-1.67043	5.26843	
90:10	TCP	1.692667	-1.77676	5.16210	
70:30	TCP	1.678000	-1.79143	5.14743	
50:50	TCP	1.371667	-2.09776	4.84110	
PDO	TCP	1.307667	-2.16176	4.77710	
TCP	ELASTIN	0.491333	-2.97810	3.96076	
90:10	PDO	0.385000	-3.08443	3.85443	
70:30	PDO	0.370333	-3.09910	3.83976	
90:10	50:50	0.321000	-3.14843	3.79043	
70:30	50:50	0.306333	-3.16310	3.77576	
50:50	PDO	0.064000	-3.40543	3.53343	
90:10	70:30	0.014667	-3.45476	3.48410	

www.manaraa.com

Oneway Analysis of Day 28 By PDO:elastin scaffolds for VEGF secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	70:30	50:50	90:10	PDO	ELASTIN	TCP	media
70:30	-2.1492	-2.1306	-1.3342	-0.8182	-0.3496	-0.2162	5.3965
50:50	-2.1306	-2.1492	-1.3529	-0.8369	-0.3682	-0.2349	5.3779
90:10	-1.3342	-1.3529	-2.1492	-1.6332	-1.1646	-1.0312	4.5815
PDO	-0.8182	-0.8369	-1.6332	-2.1492	-1.6806	-1.5472	4.0655
ELASTIN	-0.3496	-0.3682	-1.1646	-1.6806	-2.1492	-2.0159	3.5969
TCP	-0.2162	-0.2349	-1.0312	-1.5472	-2.0159	-2.1492	3.4635
media	5.3965	5.3779	4.5815	4.0655	3.5969	3.4635	-3.7226

Positive values show pairs of means that are significantly different.

Level		Mean
70:30	A	9.1790000
50:50	A	9.1603333
90:10	A	8.3640000
PDO	A	7.8480000
ELASTIN	A	7.3793333
TCP	A	7.2460000
media	B	0.7430000

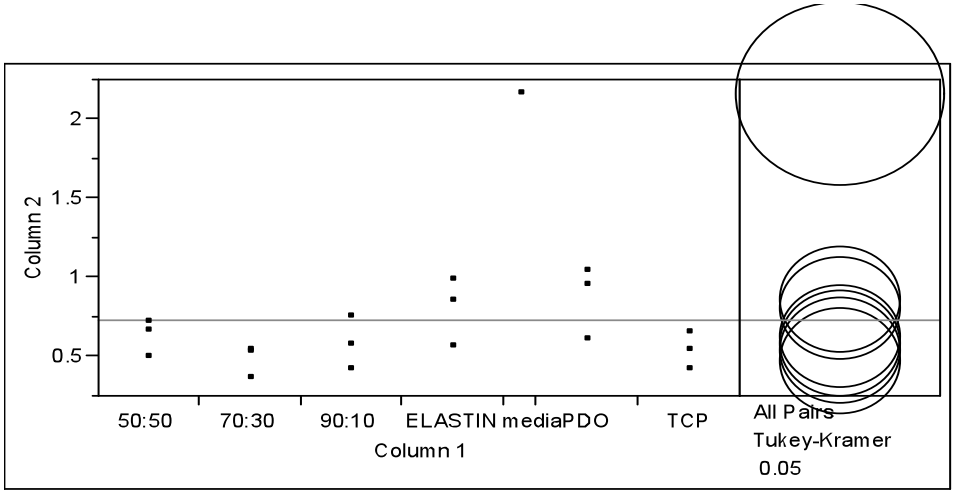
Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
70:30	media	8.436000	5.39654	11.47546	
50:50	media	8.417333	5.37788	11.45679	
90:10	media	7.621000	4.58154	10.66046	
PDO	media	7.105000	4.06554	10.14446	
ELASTIN	media	6.636333	3.59688	9.67579	
TCP	media	6.503000	3.46354	9.54246	
70:30	TCP	1.933000	-0.21622	4.08222	
50:50	TCP	1.914333	-0.23489	4.06355	
70:30	ELASTIN	1.799667	-0.34955	3.94889	
50:50	ELASTIN	1.781000	-0.36822	3.93022	

www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
70:30	PDO	1.331000	-0.81822	3.48022	
50:50	PDO	1.312333	-0.83689	3.46155	
90:10	TCP	1.118000	-1.03122	3.26722	
90:10	ELASTIN	0.984667	-1.16455	3.13389	
70:30	90:10	0.815000	-1.33422	2.96422	
50:50	90:10	0.796333	-1.35289	2.94555	
PDO	TCP	0.602000	-1.54722	2.75122	
90:10	PDO	0.516000	-1.63322	2.66522	
PDO	ELASTIN	0.468667	-1.68055	2.61789	
ELASTIN	TCP	0.133333	-2.01589	2.28255	
70:30	50:50	0.018667	-2.13055	2.16789	

Fit Y by X Group
Oneway Analysis of Day 7 By PDO:elastin scaffolds for aFGF secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	media	PDO	ELASTIN	50:50	90:10	TCP	70:30
media	-0.8157	0.6320	0.6953	0.8730	0.9147	0.9567	1.0207
PDO	0.6320	-0.4709	-0.4076	-0.2299	-0.1883	-0.1463	-0.0823
ELASTIN	0.6953	-0.4076	-0.4709	-0.2933	-0.2516	-0.2096	-0.1456
50:50	0.8730	-0.2299	-0.2933	-0.4709	-0.4293	-0.3873	-0.3233
90:10	0.9147	-0.1883	-0.2516	-0.4293	-0.4709	-0.4289	-0.3649
TCP	0.9567	-0.1463	-0.2096	-0.3873	-0.4289	-0.4709	-0.4069
70:30	1.0207	-0.0823	-0.1456	-0.3233	-0.3649	-0.4069	-0.4709

www.manaraa.com

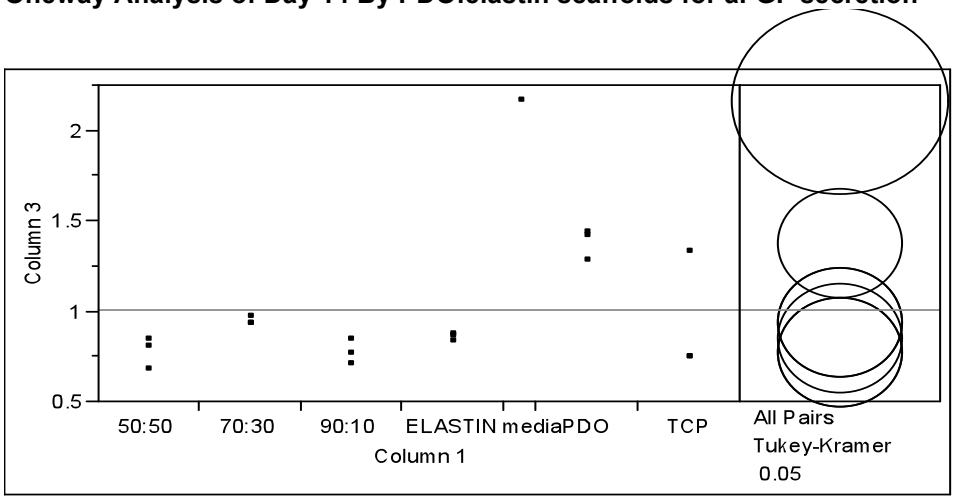
Positive values show pairs of means that are significantly different.

Level		Mean
media	A	2.1610000
PDO	B	0.8630000
ELASTIN	B	0.7996667
50:50	B	0.6220000
90:10	B	0.5803333
TCP	B	0.5383333
70:30	B	0.4743333

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
media	70:30	1.686667	1.02067	2.352660	
media	TCP	1.622667	0.95667	2.288660	
media	90:10	1.580667	0.91467	2.246660	
media	50:50	1.539000	0.87301	2.204994	
media	ELASTIN	1.361333	0.69534	2.027327	
media	PDO	1.298000	0.63201	1.963994	
PDO	70:30	0.388667	-0.08226	0.859595	
ELASTIN	70:30	0.325333	-0.14560	0.796262	
PDO	TCP	0.324667	-0.14626	0.795595	
PDO	90:10	0.282667	-0.18826	0.753595	
ELASTIN	TCP	0.261333	-0.20960	0.732262	
PDO	50:50	0.241000	-0.22993	0.711929	
ELASTIN	90:10	0.219333	-0.25160	0.690262	
ELASTIN	50:50	0.177667	-0.29326	0.648595	
50:50	70:30	0.147667	-0.32326	0.618595	
90:10	70:30	0.106000	-0.36493	0.576929	
50:50	TCP	0.083667	-0.38726	0.554595	
TCP	70:30	0.064000	-0.40693	0.534929	
PDO	ELASTIN	0.063333	-0.40760	0.534262	
90:10	TCP	0.042000	-0.42893	0.512929	
50:50	90:10	0.041667	-0.42926	0.512595	

Oneway Analysis of Day 14 By PDO:elastin scaffolds for aFGF secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

www.manaraa.com

Abs(Dif)-LSD	media	PDO	70:30	TCP	ELASTIN	50:50	90:10
media	-0.73603	0.18304	0.62104	0.62304	0.70670	0.78970	0.79037
PDO	0.18304	-0.42495	0.01305	0.01505	0.09872	0.18172	0.18239
70:30	0.62104	0.01305	-0.42495	-0.42295	-0.33928	-0.25628	-0.25561
TCP	0.62304	0.01505	-0.42295	-0.42495	-0.34128	-0.25828	-0.25761
ELASTIN	0.70670	0.09872	-0.33928	-0.34128	-0.42495	-0.34195	-0.34128
50:50	0.78970	0.18172	-0.25628	-0.25828	-0.34195	-0.42495	-0.42428
90:10	0.79037	0.18239	-0.25561	-0.25761	-0.34128	-0.42428	-0.42495

Positive values show pairs of means that are significantly different.

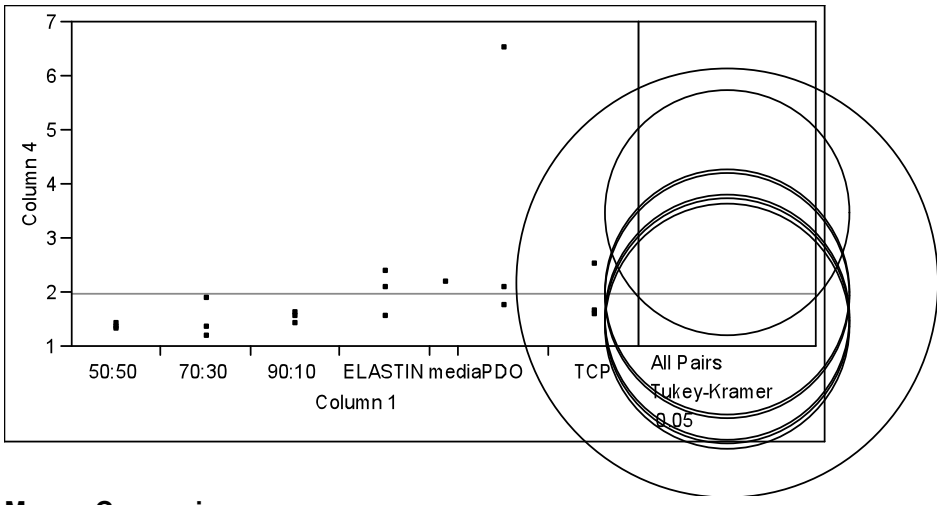
Level			Mean
media	A		2.1610000
PDO		B	1.3770000
70:30		C	0.9390000
TCP		C	0.9370000
ELASTIN		C	0.8533333
50:50		C	0.7703333
90:10		C	0.7696667

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
media	90:10	1.391333	0.790369	1.992298	
media	50:50	1.390667	0.789702	1.991631	
media	ELASTIN	1.307667	0.706702	1.908631	
media	TCP	1.224000	0.623036	1.824964	
media	70:30	1.222000	0.621036	1.822964	
media	PDO	0.784000	0.183036	1.384964	
PDO	90:10	0.607333	0.182387	1.032279	
PDO	50:50	0.606667	0.181721	1.031613	
PDO	ELASTIN	0.523667	0.098721	0.948613	
PDO	TCP	0.440000	0.015054	0.864946	
PDO	70:30	0.438000	0.013054	0.862946	
70:30	90:10	0.169333	-0.255613	0.594279	
70:30	50:50	0.168667	-0.256279	0.593613	
TCP	90:10	0.167333	-0.257613	0.592279	
TCP	50:50	0.166667	-0.258279	0.591613	
70:30	ELASTIN	0.085667	-0.339279	0.510613	
ELASTIN	90:10	0.083667	-0.341279	0.508613	
TCP	ELASTIN	0.083667	-0.341279	0.508613	
ELASTIN	50:50	0.083000	-0.341946	0.507946	
70:30	TCP	0.002000	-0.422946	0.426946	
50:50	90:10	0.000667	-0.424279	0.425613	

www.manaraa.com

Oneway Analysis of Day 21 By PDO:elastin scaffolds for aFGF secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	PDO	media	ELASTIN	TCP	90:10	70:30	50:50
PDO	-3.2311	-3.2912	-1.7745	-1.6965	-1.2975	-1.2505	-1.1391
media	-3.2912	-5.5965	-4.3912	-4.3132	-3.9142	-3.8672	-3.7559
ELASTIN	-1.7745	-4.3912	-3.2311	-3.1531	-2.7541	-2.7071	-2.5958
TCP	-1.6965	-4.3132	-3.1531	-3.2311	-2.8321	-2.7851	-2.6738
90:10	-1.2975	-3.9142	-2.7541	-2.8321	-3.2311	-3.1841	-3.0728
70:30	-1.2505	-3.8672	-2.7071	-2.7851	-3.1841	-3.2311	-3.1198
50:50	-1.1391	-3.7559	-2.5958	-2.6738	-3.0728	-3.1198	-3.2311

Positive values show pairs of means that are significantly different.

Level		Mean
PDO	A	3.4393333
media	A	2.1610000
ELASTIN	A	1.9826667
TCP	A	1.9046667
90:10	A	1.5056667
70:30	A	1.4586667
50:50	A	1.3473333

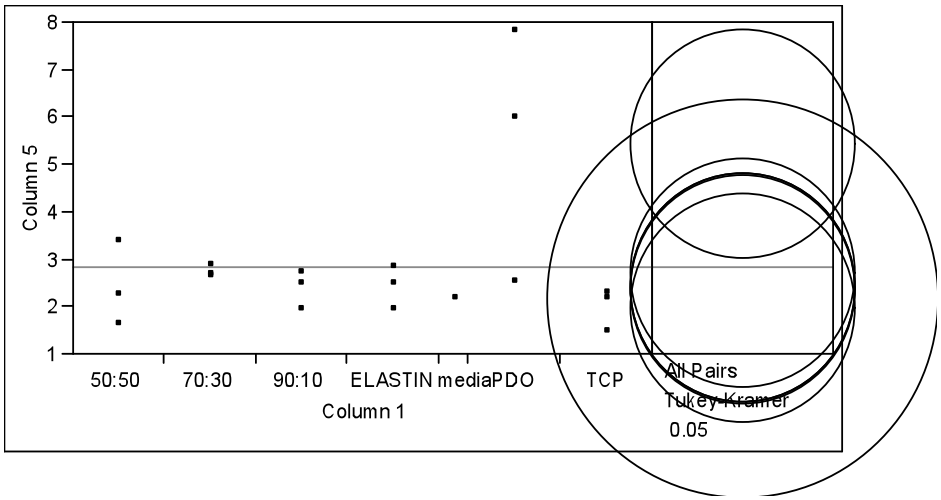
Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
PDO	50:50	2.092000	-1.13914	5.323138	
PDO	70:30	1.980667	-1.25047	5.211804	
PDO	90:10	1.933667	-1.29747	5.164804	
PDO	TCP	1.534667	-1.69647	4.765804	
PDO	ELASTIN	1.456667	-1.77447	4.687804	
PDO	media	1.278333	-3.29119	5.847852	
media	50:50	0.813667	-3.75585	5.383186	
media	70:30	0.702333	-3.86719	5.271852	
media	90:10	0.655333	-3.91419	5.224852	
ELASTIN	50:50	0.635333	-2.59580	3.866471	
TCP	50:50	0.557333	-2.67380	3.788471	
ELASTIN	70:30	0.524000	-2.70714	3.755138	

www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
ELASTIN	90:10	0.477000	-2.75414	3.708138	
TCP	70:30	0.446000	-2.78514	3.677138	
TCP	90:10	0.399000	-2.83214	3.630138	
media	TCP	0.256333	-4.31319	4.825852	
media	ELASTIN	0.178333	-4.39119	4.747852	
90:10	50:50	0.158333	-3.07280	3.389471	
70:30	50:50	0.111333	-3.11980	3.342471	
ELASTIN	TCP	0.078000	-3.15314	3.309138	
90:10	70:30	0.047000	-3.18414	3.278138	

Oneway Analysis of Day 28 By PDO:elastin scaffolds for aFGF secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	PDO	70:30	50:50	ELASTIN	90:10	media	TCP
PDO	-3.4183	-0.7043	-0.3963	-0.3937	-0.3523	-1.5612	0.0503
70:30	-0.7043	-3.4183	-3.1103	-3.1077	-3.0663	-4.2752	-2.6637
50:50	-0.3963	-3.1103	-3.4183	-3.4157	-3.3743	-4.5832	-2.9717
ELASTIN	-0.3937	-3.1077	-3.4157	-3.4183	-3.3770	-4.5859	-2.9743
90:10	-0.3523	-3.0663	-3.3743	-3.3770	-3.4183	-4.6272	-3.0157
media	-1.5612	-4.2752	-4.5832	-4.5859	-4.6272	-5.9207	-4.6386
TCP	0.0503	-2.6637	-2.9717	-2.9743	-3.0157	-4.6386	-3.4183

Positive values show pairs of means that are significantly different.

Level			Mean
PDO	A		5.4340000
70:30	A	B	2.7200000
50:50	A	B	2.4120000
ELASTIN	A	B	2.4093333
90:10	A	B	2.3680000
media	A	B	2.1610000
TCP		B	1.9653333

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
PDO	TCP	3.468667	0.05034	6.886989	

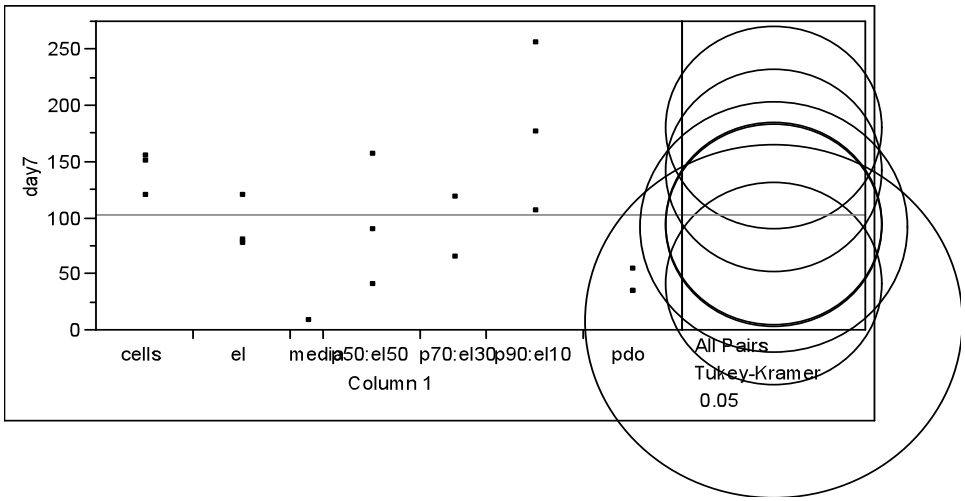
www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
PDO	media	3.273000	-1.56124	8.107237	
PDO	90:10	3.066000	-0.35232	6.484322	
PDO	ELASTIN	3.024667	-0.39366	6.442989	
PDO	50:50	3.022000	-0.39632	6.440322	
PDO	70:30	2.714000	-0.70432	6.132322	
70:30	TCP	0.754667	-2.66366	4.172989	
70:30	media	0.559000	-4.27524	5.393237	
50:50	TCP	0.446667	-2.97166	3.864989	
ELASTIN	TCP	0.444000	-2.97432	3.862322	
90:10	TCP	0.402667	-3.01566	3.820989	
70:30	90:10	0.352000	-3.06632	3.770322	
70:30	ELASTIN	0.310667	-3.10766	3.728989	
70:30	50:50	0.308000	-3.11032	3.726322	
50:50	media	0.251000	-4.58324	5.085237	
ELASTIN	media	0.248333	-4.58590	5.082570	
90:10	media	0.207000	-4.62724	5.041237	
media	TCP	0.195667	-4.63857	5.029904	
50:50	90:10	0.044000	-3.37432	3.462322	
ELASTIN	90:10	0.041333	-3.37699	3.459655	
50:50	ELASTIN	0.002667	-3.41566	3.420989	

www.manaraa.com

Fit Y by X Group

Oneway Analysis of day 7 by PDO:Elastin scaffolds for TGF-β1 secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.55539	0.05

Abs(Dif)-LSD	p90:el10	cells	p50:el50	el	p70:el30	pdo	media
p90:el10	-128.50	-90.95	-43.96	-41.38	-56.04	10.35	-11.09
cells	-90.95	-128.50	-81.52	-78.93	-93.60	-27.21	-48.65
p50:el50	-43.96	-81.52	-128.50	-125.92	-140.58	-74.20	-95.64
el	-41.38	-78.93	-125.92	-128.50	-143.17	-76.78	-98.22
p70:el30	-56.04	-93.60	-140.58	-143.17	-157.38	-92.45	-109.75
pdo	10.35	-27.21	-74.20	-76.78	-92.45	-128.50	-149.94
media	-11.09	-48.65	-95.64	-98.22	-109.75	-149.94	-222.58

Positive values show pairs of means that are significantly different.

Level			Mean
p90:el10	A		178.93900
cells	A	B	141.38333
p50:el50	A	B	94.39567
el	A	B	91.81267
p70:el30	A	B	91.30700
pdo		B	40.08867
media	A	B	8.30000

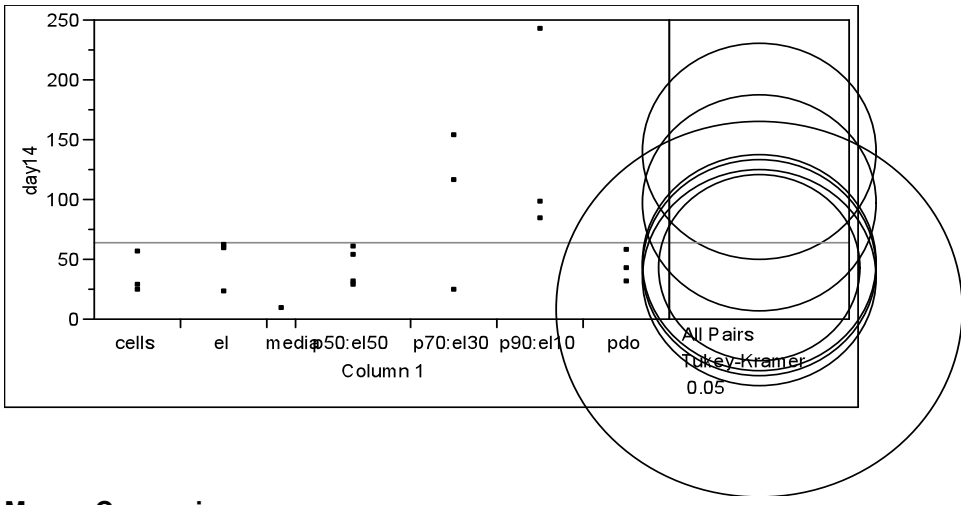
Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
p90:el10	media	170.6390	-11.093	352.3715	
p90:el10	pdo	138.8503	10.346	267.3546	
cells	media	133.0833	-48.649	314.8158	
cells	pdo	101.2947	-27.210	229.7989	
p90:el10	p70:el30	87.6320	-56.040	231.3041	
p90:el10	el	87.1263	-41.378	215.6306	
p50:el50	media	86.0957	-95.637	267.8281	
p90:el10	p50:el50	84.5433	-43.961	213.0476	
el	media	83.5127	-98.220	265.2451	

www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
p70:el30	media	83.0070	-109.749	275.7634	
p50:el50	pdo	54.3070	-74.197	182.8113	
el	pdo	51.7240	-76.780	180.2283	
p70:el30	pdo	51.2183	-92.454	194.8905	
cells	p70:el30	50.0763	-93.596	193.7485	
cells	el	49.5707	-78.934	178.0749	
cells	p50:el50	46.9877	-81.517	175.4919	
p90:el10	cells	37.5557	-90.949	166.0599	
pdo	media	31.7887	-149.944	213.5211	
p50:el50	p70:el30	3.0887	-140.583	146.7608	
p50:el50	el	2.5830	-125.921	131.0873	
el	p70:el30	0.5057	-143.166	144.1778	

Oneway Analysis of day 14 by PDO:elastin scaffolds for TGF-β1 secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.45353	0.05

Abs(Dif)-LSD	p90:el10	p70:el30	el	pdo	p50:el50	cells	media
p90:el10	-127.98	-84.32	-34.58	-30.44	-21.80	-22.36	-48.56
p70:el30	-84.32	-127.98	-78.24	-74.10	-65.46	-66.02	-92.22
el	-34.58	-78.24	-127.98	-123.84	-115.19	-115.76	-141.96
pdo	-30.44	-74.10	-123.84	-127.98	-119.33	-119.90	-146.10
p50:el50	-21.80	-65.46	-115.19	-119.33	-110.83	-112.01	-140.73
cells	-22.36	-66.02	-115.76	-119.90	-112.01	-127.98	-154.18
media	-48.56	-92.22	-141.96	-146.10	-140.73	-154.18	-221.67

Positive values show pairs of means that are significantly different.

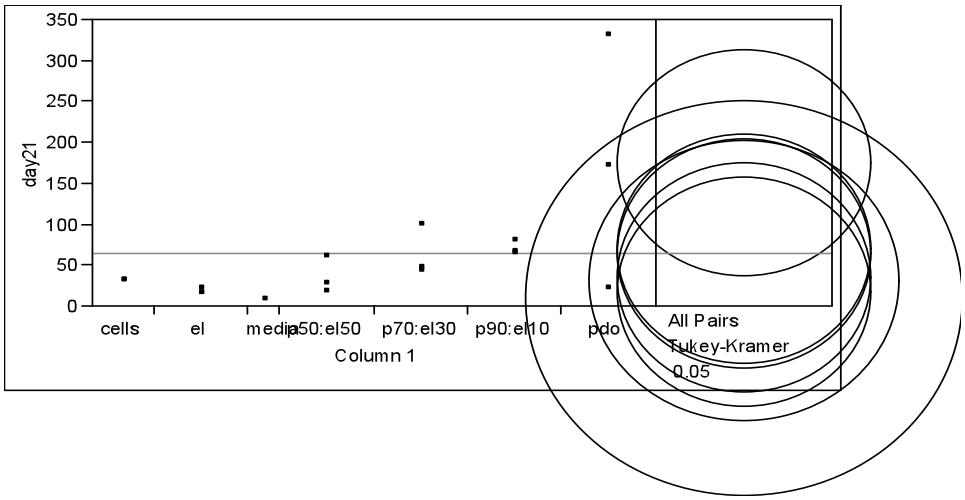
Level		Mean
p90:el10	A	140.72967
p70:el30	A	97.06933
el	A	47.33267
pdo	A	43.19333
p50:el50	A	42.81275
cells	A	35.11067
media	A	8.30000

Levels not connected by same letter are significantly different.

www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
p90:el10	media	132.4297	-48.561	313.4203	
p90:el10	cells	105.6190	-22.361	233.5987	
p90:el10	p50:el50	97.9169	-21.797	217.6310	
p90:el10	pdo	97.5363	-30.443	225.5160	
p90:el10	el	93.3970	-34.583	221.3767	
p70:el30	media	88.7693	-92.221	269.7600	
p70:el30	cells	61.9587	-66.021	189.9384	
p70:el30	p50:el50	54.2566	-65.457	173.9706	
p70:el30	pdo	53.8760	-74.104	181.8557	
p70:el30	el	49.7367	-78.243	177.7164	
p90:el10	p70:el30	43.6603	-84.319	171.6400	
el	media	39.0327	-141.958	220.0233	
pdo	media	34.8933	-146.097	215.8840	
p50:el50	media	34.5128	-140.731	209.7562	
cells	media	26.8107	-154.180	207.8013	
el	cells	12.2220	-115.758	140.2017	
pdo	cells	8.0827	-119.897	136.0624	
p50:el50	cells	7.7021	-112.012	127.4161	
el	p50:el50	4.5199	-115.194	124.2340	
el	pdo	4.1393	-123.840	132.1190	
pdo	p50:el50	0.3806	-119.333	120.0946	

Oneway Analysis of day 21 by PDO:elastin scaffolds for TGF-β1 secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.55539	0.05

Abs(Dif)-LSD	pdo	p90:el10	p70:el30	p50:el50	cells	el	media
pdo	-197.56	-93.88	-87.19	-58.69	-77.98	-41.48	-113.78
p90:el10	-93.88	-197.56	-190.87	-162.37	-181.67	-145.16	-217.47
p70:el30	-87.19	-190.87	-197.56	-169.06	-188.36	-151.85	-224.16
p50:el50	-58.69	-162.37	-169.06	-197.56	-216.86	-180.35	-252.66
cells	-77.98	-181.67	-188.36	-216.86	-241.96	-207.69	-273.63
el	-41.48	-145.16	-151.85	-180.35	-207.69	-197.56	-269.87
media	-113.78	-217.47	-224.16	-252.66	-273.63	-269.87	-342.19

Positive values show pairs of means that are significantly different.

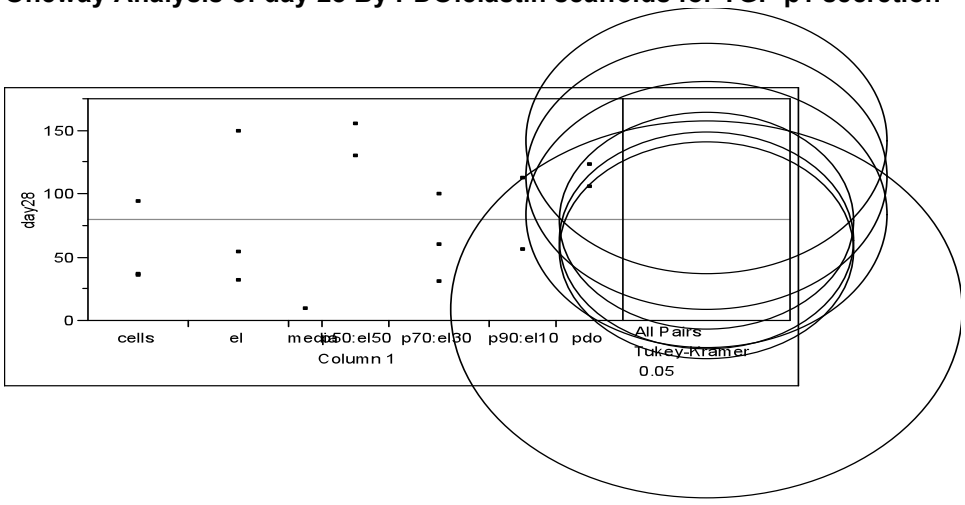
www.manaraa.com

Level		Mean
pdo	A	173.91267
p90:el10	A	70.22733
p70:el30	A	63.53767
p50:el50	A	35.03833
cells	A	31.01650
el	A	17.82833
media	A	8.30000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
pdo	media	165.6127	-113.781	445.0062	
pdo	el	156.0843	-41.477	353.6454	
pdo	cells	142.8962	-77.984	363.7761	
pdo	p50:el50	138.8743	-58.687	336.4354	
pdo	p70:el30	110.3750	-87.186	307.9360	
pdo	p90:el10	103.6853	-93.876	301.2464	
p90:el10	media	61.9273	-217.466	341.3208	
p70:el30	media	55.2377	-224.156	334.6312	
p90:el10	el	52.3990	-145.162	249.9600	
p70:el30	el	45.7093	-151.852	243.2704	
p90:el10	cells	39.2108	-181.669	260.0908	
p90:el10	p50:el50	35.1890	-162.372	232.7500	
p70:el30	cells	32.5212	-188.359	253.4011	
p70:el30	p50:el50	28.4993	-169.062	226.0604	
p50:el50	media	26.7383	-252.655	306.1318	
cells	media	22.7165	-273.625	319.0581	
p50:el50	el	17.2100	-180.351	214.7710	
cells	el	13.1882	-207.692	234.0681	
el	media	9.5283	-269.865	288.9218	
p90:el10	p70:el30	6.6897	-190.871	204.2507	
p50:el50	cells	4.0218	-216.858	224.9018	

Oneway Analysis of day 28 By PDO:elastin scaffolds for TGF-β1 secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.70826	0.05

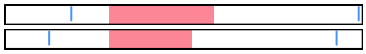
Abs(Dif)-LSD	p50:el50	pdo	p90:el10	el	p70:el30	cells	media
p50:el50	-149.00	-120.67	-90.68	-71.89	-57.03	-48.92	-48.87
pdo	-120.67	-149.00	-119.01	-100.23	-85.36	-77.25	-77.20
p90:el10	-90.68	-119.01	-149.00	-130.22	-115.35	-107.24	-107.19
el	-71.89	-100.23	-130.22	-121.66	-106.80	-98.69	-102.55
p70:el30	-57.03	-85.36	-115.35	-106.80	-121.66	-113.55	-117.42

www.manaraa.com

Positive values show pairs of means that are significantly different.

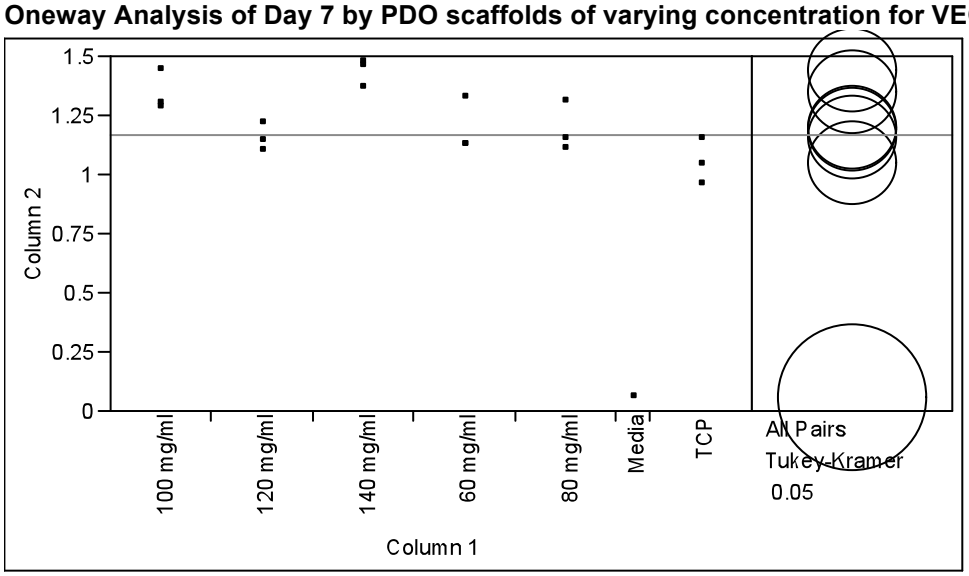
Level		Mean
p50:el50	A	141.92300
pdo	A	113.59200
p90:el10	A	83.60000
el	A	77.79867
p70:el30	A	62.93600
cells	A	54.82500
media	A	8.30000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
p50:el50	media	133.6230	-48.866	316.1116	
pdo	media	105.2920	-77.197	287.7806	
p50:el50	cells	87.0980	-48.921	223.1169	
p50:el50	p70:el30	78.9870	-57.032	215.0059	
p90:el10	media	75.3000	-107.189	257.7886	
el	media	69.4987	-102.553	241.5505	
p50:el50	el	64.1243	-71.895	200.1433	
pdo	cells	58.7670	-77.252	194.7859	
p50:el50	p90:el10	58.3230	-90.678	207.3243	
p70:el30	media	54.6360	-117.416	226.6879	
pdo	p70:el30	50.6560	-85.363	186.6749	
cells	media	46.5250	-125.527	218.5769	
pdo	el	35.7933	-100.226	171.8123	
pdo	p90:el10	29.9920	-119.009	178.9933	
p90:el10	cells	28.7750	-107.244	164.7939	
p50:el50	pdo	28.3310	-120.670	177.3323	
el	cells	22.9737	-98.685	144.6327	
p90:el10	p70:el30	20.6640	-115.355	156.6829	
el	p70:el30	14.8627	-106.796	136.5217	
p70:el30	cells	8.1110	-113.548	129.7700	
p90:el10	el	5.8013	-130.218	141.8203	

www.manaraa.com

Fit Y by X Group
Oneway Analysis of Day 7 by PDO scaffolds of varying concentration for VEGF secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	140 mg/ml	100 mg/ml	60 mg/ml	80 mg/ml	120 mg/ml	TCP	Media
140 mg/ml	-0.2537	-0.1633	-0.0127	-0.0080	0.0290	0.1313	1.0149
100 mg/ml	-0.1633	-0.2537	-0.1030	-0.0983	-0.0613	0.0410	0.9246
60 mg/ml	-0.0127	-0.1030	-0.2537	-0.2490	-0.2120	-0.1097	0.7739
80 mg/ml	-0.0080	-0.0983	-0.2490	-0.2537	-0.2167	-0.1143	0.7692
120 mg/ml	0.0290	-0.0613	-0.2120	-0.2167	-0.2537	-0.1513	0.7322
TCP	0.1313	0.0410	-0.1097	-0.1143	-0.1513	-0.2537	0.6299
Media	1.0149	0.9246	0.7739	0.7692	0.7322	0.6299	-0.4394

Positive values show pairs of means that are significantly different.

Level					Mean
140 mg/ml	A				1.4336667
100 mg/ml	A	B			1.3433333
60 mg/ml	A	B	C		1.1926667
80 mg/ml	A	B	C		1.1880000
120 mg/ml		B	C		1.1510000
TCP			C		1.0486667
Media				D	0.0600000

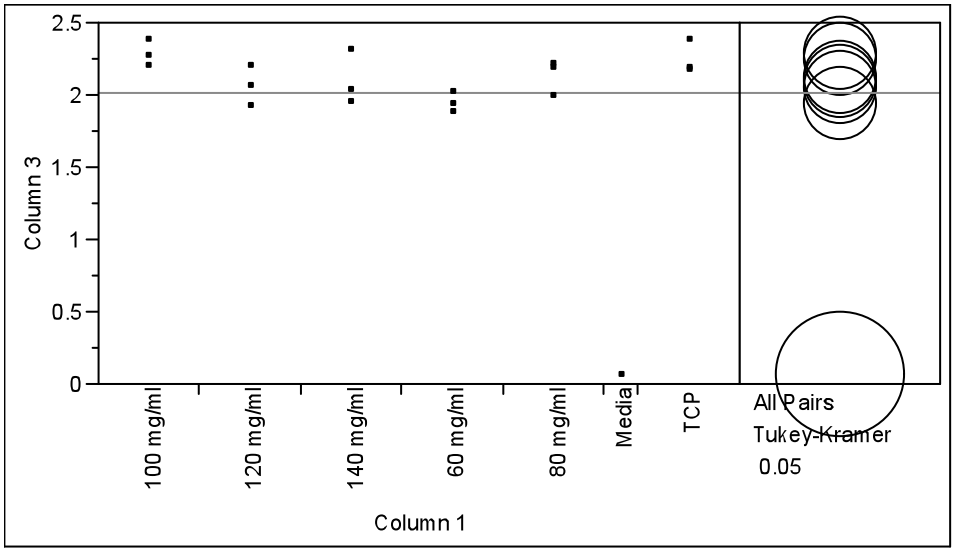
Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
140 mg/ml	Media	1.373667	1.01491	1.732425	
100 mg/ml	Media	1.283333	0.92457	1.642092	
60 mg/ml	Media	1.132667	0.77391	1.491425	
80 mg/ml	Media	1.128000	0.76924	1.486759	
120 mg/ml	Media	1.091000	0.73224	1.449759	

www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
TCP	Media	0.988667	0.62991	1.347425	
140 mg/ml	TCP	0.385000	0.13132	0.638681	
100 mg/ml	TCP	0.294667	0.04099	0.548347	
140 mg/ml	120 mg/ml	0.282667	0.02899	0.536347	
140 mg/ml	80 mg/ml	0.245667	-0.00801	0.499347	
140 mg/ml	60 mg/ml	0.241000	-0.01268	0.494681	
100 mg/ml	120 mg/ml	0.192333	-0.06135	0.446014	
100 mg/ml	80 mg/ml	0.155333	-0.09835	0.409014	
100 mg/ml	60 mg/ml	0.150667	-0.10301	0.404347	
60 mg/ml	TCP	0.144000	-0.10968	0.397681	
80 mg/ml	TCP	0.139333	-0.11435	0.393014	
120 mg/ml	TCP	0.102333	-0.15135	0.356014	
140 mg/ml	100 mg/ml	0.090333	-0.16335	0.344014	
60 mg/ml	120 mg/ml	0.041667	-0.21201	0.295347	
80 mg/ml	120 mg/ml	0.037000	-0.21668	0.290681	
60 mg/ml	80 mg/ml	0.004667	-0.24901	0.258347	

Oneway Analysis of Day 14 by PDO scaffolds of varying concentration for VEGF secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	100 mg/ml	TCP	80 mg/ml	140 mg/ml	120 mg/ml	60 mg/ml	Media
100 mg/ml	-0.3592	-0.3205	-0.2038	-0.1745	-0.1332	-0.0175	1.7144
TCP	-0.3205	-0.3592	-0.2425	-0.2132	-0.1718	-0.0562	1.6757
80 mg/ml	-0.2038	-0.2425	-0.3592	-0.3298	-0.2885	-0.1728	1.5590
140 mg/ml	-0.1745	-0.2132	-0.3298	-0.3592	-0.3178	-0.2022	1.5297
120 mg/ml	-0.1332	-0.1718	-0.2885	-0.3178	-0.3592	-0.2435	1.4884
60 mg/ml	-0.0175	-0.0562	-0.1728	-0.2022	-0.2435	-0.3592	1.3727
Media	1.7144	1.6757	1.5590	1.5297	1.4884	1.3727	-0.6221

Positive values show pairs of means that are significantly different.

Level		Mean
100 mg/ml	A	2.2823333

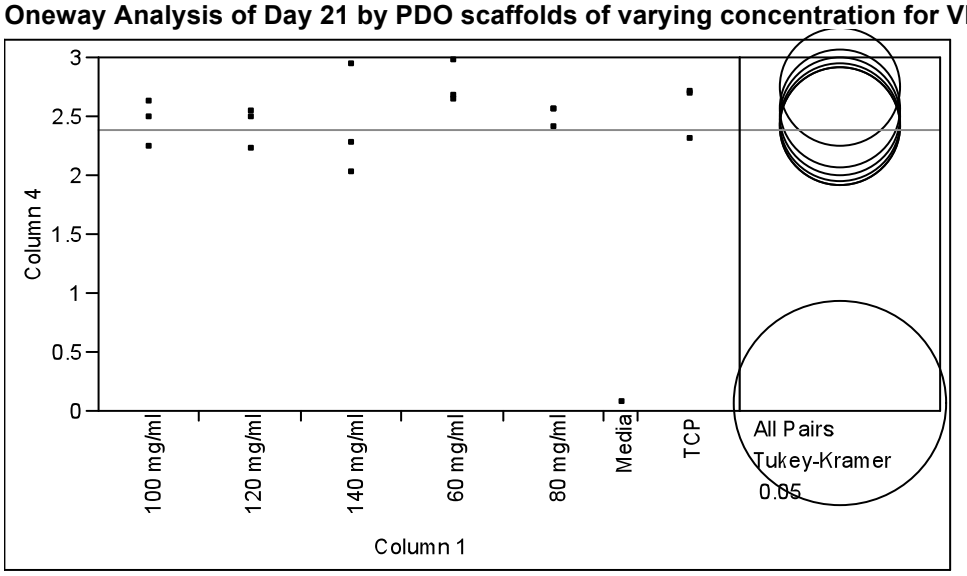
www.manaraa.com

Level		Mean
TCP	A	2.2436667
80 mg/ml	A	2.1270000
140 mg/ml	A	2.0976667
120 mg/ml	A	2.0563333
60 mg/ml	A	1.9406667
Media	B	0.0600000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
100 mg/ml	Media	2.222333	1.71438	2.730290	
TCP	Media	2.183667	1.67571	2.691623	
80 mg/ml	Media	2.067000	1.55904	2.574956	
140 mg/ml	Media	2.037667	1.52971	2.545623	
120 mg/ml	Media	1.996333	1.48838	2.504290	
60 mg/ml	Media	1.880667	1.37271	2.388623	
100 mg/ml	60 mg/ml	0.341667	-0.01751	0.700846	
TCP	60 mg/ml	0.303000	-0.05618	0.662179	
100 mg/ml	120 mg/ml	0.226000	-0.13318	0.585179	
TCP	120 mg/ml	0.187333	-0.17185	0.546513	
80 mg/ml	60 mg/ml	0.186333	-0.17285	0.545513	
100 mg/ml	140 mg/ml	0.184667	-0.17451	0.543846	
140 mg/ml	60 mg/ml	0.157000	-0.20218	0.516179	
100 mg/ml	80 mg/ml	0.155333	-0.20385	0.514513	
TCP	140 mg/ml	0.146000	-0.21318	0.505179	
TCP	80 mg/ml	0.116667	-0.24251	0.475846	
120 mg/ml	60 mg/ml	0.115667	-0.24351	0.474846	
80 mg/ml	120 mg/ml	0.070667	-0.28851	0.429846	
140 mg/ml	120 mg/ml	0.041333	-0.31785	0.400513	
100 mg/ml	TCP	0.038667	-0.32051	0.397846	
80 mg/ml	140 mg/ml	0.029333	-0.32985	0.388513	

Oneway Analysis of Day 21 by PDO scaffolds of varying concentration for VEGF secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

www.manaraa.com

Abs(Dif)-LSD	60 mg/ml	TCP	80 mg/ml	100 mg/ml	120 mg/ml	140 mg/ml	Media
60 mg/ml	-0.7145	-0.5218	-0.4635	-0.4088	-0.3732	-0.3678	1.6796
TCP	-0.5218	-0.7145	-0.6562	-0.6015	-0.5658	-0.5605	1.4869
80 mg/ml	-0.4635	-0.6562	-0.7145	-0.6598	-0.6242	-0.6188	1.4286
100 mg/ml	-0.4088	-0.6015	-0.6598	-0.7145	-0.6788	-0.6735	1.3739
120 mg/ml	-0.3732	-0.5658	-0.6242	-0.6788	-0.7145	-0.7092	1.3382
140 mg/ml	-0.3678	-0.5605	-0.6188	-0.6735	-0.7092	-0.7145	1.3329
Media	1.6796	1.4869	1.4286	1.3739	1.3382	1.3329	-1.2375

Positive values show pairs of means that are significantly different.

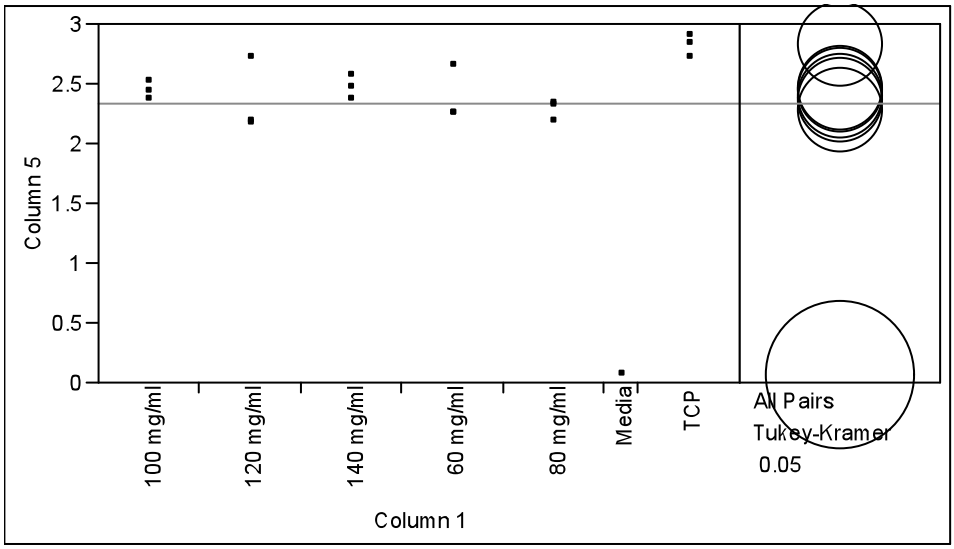
Level		Mean
60 mg/ml	A	2.7500000
TCP	A	2.5573333
80 mg/ml	A	2.4990000
100 mg/ml	A	2.4443333
120 mg/ml	A	2.4086667
140 mg/ml	A	2.4033333
Media	B	0.0600000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
60 mg/ml	Media	2.690000	1.67955	3.700449	
TCP	Media	2.497333	1.48688	3.507783	
80 mg/ml	Media	2.439000	1.42855	3.449449	
100 mg/ml	Media	2.384333	1.37388	3.394783	
120 mg/ml	Media	2.348667	1.33822	3.359116	
140 mg/ml	Media	2.343333	1.33288	3.353783	
60 mg/ml	140 mg/ml	0.346667	-0.36783	1.061162	
60 mg/ml	120 mg/ml	0.341333	-0.37316	1.055829	
60 mg/ml	100 mg/ml	0.305667	-0.40883	1.020162	
60 mg/ml	80 mg/ml	0.251000	-0.46350	0.965496	
60 mg/ml	TCP	0.192667	-0.52183	0.907162	
TCP	140 mg/ml	0.154000	-0.56050	0.868496	
TCP	120 mg/ml	0.148667	-0.56583	0.863162	
TCP	100 mg/ml	0.113000	-0.60150	0.827496	
80 mg/ml	140 mg/ml	0.095667	-0.61883	0.810162	
80 mg/ml	120 mg/ml	0.090333	-0.62416	0.804829	
TCP	80 mg/ml	0.058333	-0.65616	0.772829	
80 mg/ml	100 mg/ml	0.054667	-0.65983	0.769162	
100 mg/ml	140 mg/ml	0.041000	-0.67350	0.755496	
100 mg/ml	120 mg/ml	0.035667	-0.67883	0.750162	
120 mg/ml	140 mg/ml	0.005333	-0.70916	0.719829	

www.manaraa.com

Oneway Analysis of Day 28 by PDO scaffolds of varying concentration for VEGF secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	TCP	140 mg/ml	100 mg/ml	60 mg/ml	120 mg/ml	80 mg/ml	Media
TCP	-0.5027	-0.1447	-0.1207	-0.0687	-0.0370	0.0443	2.0511
140 mg/ml	-0.1447	-0.5027	-0.4787	-0.4267	-0.3950	-0.3137	1.6931
100 mg/ml	-0.1207	-0.4787	-0.5027	-0.4507	-0.4190	-0.3377	1.6691
60 mg/ml	-0.0687	-0.4267	-0.4507	-0.5027	-0.4710	-0.3897	1.6171
120 mg/ml	-0.0370	-0.3950	-0.4190	-0.4710	-0.5027	-0.4213	1.5854
80 mg/ml	0.0443	-0.3137	-0.3377	-0.3897	-0.4213	-0.5027	1.5041
Media	2.0511	1.6931	1.6691	1.6171	1.5854	1.5041	-0.8707

Positive values show pairs of means that are significantly different.

Level				Mean
TCP	A			2.8220000
140 mg/ml	A	B		2.4640000
100 mg/ml	A	B		2.4400000
60 mg/ml	A	B		2.3880000
120 mg/ml	A	B		2.3563333
80 mg/ml		B		2.2750000
Media			C	0.0600000

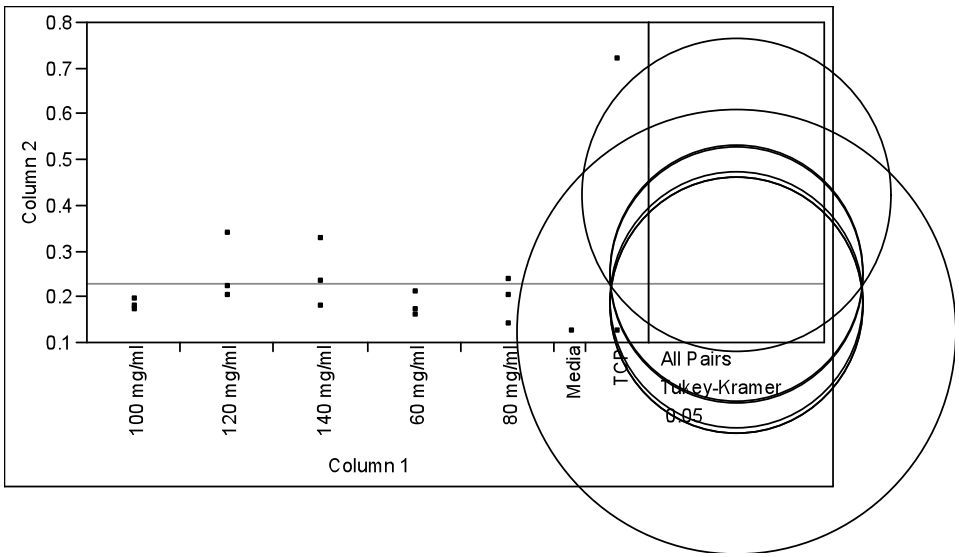
Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
TCP	Media	2.762000	2.05110	3.472895	
140 mg/ml	Media	2.404000	1.69310	3.114895	
100 mg/ml	Media	2.380000	1.66910	3.090895	
60 mg/ml	Media	2.328000	1.61710	3.038895	
120 mg/ml	Media	2.296333	1.58544	3.007229	
80 mg/ml	Media	2.215000	1.50410	2.925895	
TCP	80 mg/ml	0.547000	0.04432	1.049679	
TCP	120 mg/ml	0.465667	-0.03701	0.968346	

www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
TCP	60 mg/ml	0.434000	-0.06868	0.936679	
TCP	100 mg/ml	0.382000	-0.12068	0.884679	
TCP	140 mg/ml	0.358000	-0.14468	0.860679	
140 mg/ml	80 mg/ml	0.189000	-0.31368	0.691679	
100 mg/ml	80 mg/ml	0.165000	-0.33768	0.667679	
60 mg/ml	80 mg/ml	0.113000	-0.38968	0.615679	
140 mg/ml	120 mg/ml	0.107667	-0.39501	0.610346	
100 mg/ml	120 mg/ml	0.083667	-0.41901	0.586346	
120 mg/ml	80 mg/ml	0.081333	-0.42135	0.584012	
140 mg/ml	60 mg/ml	0.076000	-0.42668	0.578679	
100 mg/ml	60 mg/ml	0.052000	-0.45068	0.554679	
60 mg/ml	120 mg/ml	0.031667	-0.47101	0.534346	
140 mg/ml	100 mg/ml	0.024000	-0.47868	0.526679	

Fit Y by X Group
Oneway Analysis of Day 7 by PDO scaffolds of varying concentration for aFGF secretion



Missing Rows
1

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.55539	0.05

Abs(Dif)-LSD	TCP	120 mg/ml	140 mg/ml	80 mg/ml	60 mg/ml	100 mg/ml	Media
TCP	-0.48520	-0.27559	-0.26892	-0.21492	-0.20292	-0.20259	-0.29824
120 mg/ml	-0.27559	-0.39616	-0.38950	-0.33550	-0.32350	-0.32316	-0.43159
140 mg/ml	-0.26892	-0.38950	-0.39616	-0.34216	-0.33016	-0.32983	-0.43826
80 mg/ml	-0.21492	-0.33550	-0.34216	-0.39616	-0.38416	-0.38383	-0.49226
60 mg/ml	-0.20292	-0.32350	-0.33016	-0.38416	-0.39616	-0.39583	-0.50426
100 mg/ml	-0.20259	-0.32316	-0.32983	-0.38383	-0.39583	-0.39616	-0.50459
Media	-0.29824	-0.43159	-0.43826	-0.49226	-0.50426	-0.50459	-0.68617

Positive values show pairs of means that are significantly different.

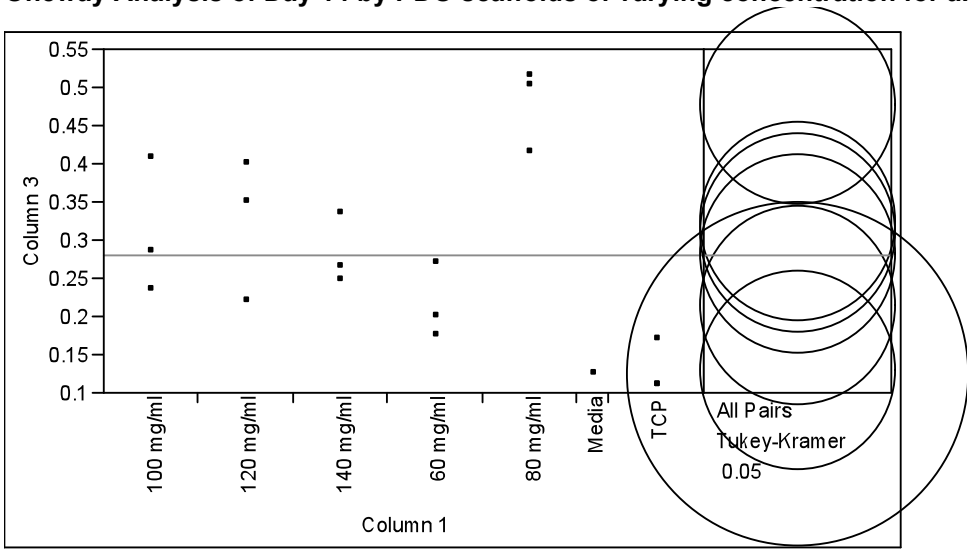
www.manaraa.com

Level		Mean
TCP	A	0.42000000
120 mg/ml	A	0.25266667
140 mg/ml	A	0.24600000
80 mg/ml	A	0.19200000
60 mg/ml	A	0.18000000
100 mg/ml	A	0.17966667
Media	A	0.12400000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
TCP	Media	0.2960000	-0.298243	0.8902432	
TCP	100 mg/ml	0.2403333	-0.202589	0.6832560	
TCP	60 mg/ml	0.2400000	-0.202923	0.6829227	
TCP	80 mg/ml	0.2280000	-0.214923	0.6709227	
TCP	140 mg/ml	0.1740000	-0.268923	0.6169227	
TCP	120 mg/ml	0.1673333	-0.275589	0.6102560	
120 mg/ml	Media	0.1286667	-0.431591	0.6889245	
140 mg/ml	Media	0.1220000	-0.438258	0.6822578	
120 mg/ml	100 mg/ml	0.0730000	-0.323162	0.4691621	
120 mg/ml	60 mg/ml	0.0726667	-0.323495	0.4688288	
80 mg/ml	Media	0.0680000	-0.492258	0.6282578	
140 mg/ml	100 mg/ml	0.0663333	-0.329829	0.4624954	
140 mg/ml	60 mg/ml	0.0660000	-0.330162	0.4621621	
120 mg/ml	80 mg/ml	0.0606667	-0.335495	0.4568288	
60 mg/ml	Media	0.0560000	-0.504258	0.6162578	
100 mg/ml	Media	0.0556667	-0.504591	0.6159245	
140 mg/ml	80 mg/ml	0.0540000	-0.342162	0.4501621	
80 mg/ml	100 mg/ml	0.0123333	-0.383829	0.4084954	
80 mg/ml	60 mg/ml	0.0120000	-0.384162	0.4081621	
120 mg/ml	140 mg/ml	0.0066667	-0.389495	0.4028288	
60 mg/ml	100 mg/ml	0.0003333	-0.395829	0.3964954	

Oneway Analysis of Day 14 by PDO scaffolds of varying concentration for aFGF secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

www.manaraa.com

q* **Alpha**

Abs(Dif)-LSD	80 mg/ml	120 mg/ml	100 mg/ml	140 mg/ml	60 mg/ml	TCP	Media
80 mg/ml	-0.18488	-0.03221	-0.01654	0.00979	0.07679	0.16212	0.09155
120 mg/ml	-0.03221	-0.18488	-0.16921	-0.14288	-0.07588	0.00946	-0.06112
100 mg/ml	-0.01654	-0.16921	-0.18488	-0.15854	-0.09154	-0.00621	-0.07679
140 mg/ml	0.00979	-0.14288	-0.15854	-0.18488	-0.11788	-0.03254	-0.10312
60 mg/ml	0.07679	-0.07588	-0.09154	-0.11788	-0.18488	-0.09954	-0.17012
TCP	0.16212	0.00946	-0.00621	-0.03254	-0.09954	-0.18488	-0.25545
Media	0.09155	-0.06112	-0.07679	-0.10312	-0.17012	-0.25545	-0.32021

Positive values show pairs of means that are significantly different.

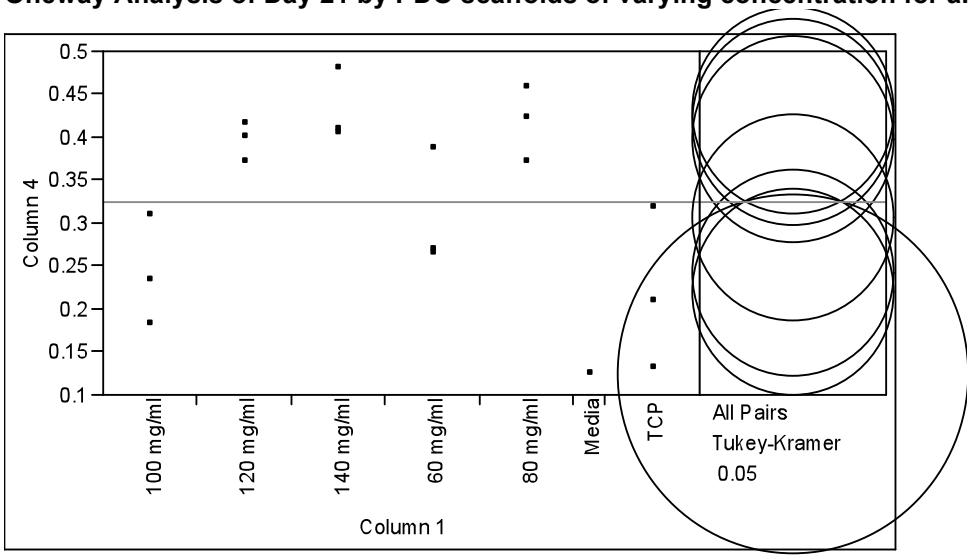
Level				Mean
80 mg/ml	A			0.47700000
120 mg/ml	A	B		0.32433333
100 mg/ml	A	B	C	0.30866667
140 mg/ml		B	C	0.28233333
60 mg/ml		B	C	0.21533333
TCP			C	0.13000000
Media		B	C	0.12400000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
80 mg/ml	Media	0.3530000	0.091546	0.6144540	
80 mg/ml	TCP	0.3470000	0.162124	0.5318759	
80 mg/ml	60 mg/ml	0.2616667	0.076791	0.4465426	
120 mg/ml	Media	0.2003333	-0.061121	0.4617873	
80 mg/ml	140 mg/ml	0.1946667	0.009791	0.3795426	
120 mg/ml	TCP	0.1943333	0.009457	0.3792092	
100 mg/ml	Media	0.1846667	-0.076787	0.4461207	
100 mg/ml	TCP	0.1786667	-0.006209	0.3635426	
80 mg/ml	100 mg/ml	0.1683333	-0.016543	0.3532092	
140 mg/ml	Media	0.1583333	-0.103121	0.4197873	
80 mg/ml	120 mg/ml	0.1526667	-0.032209	0.3375426	
140 mg/ml	TCP	0.1523333	-0.032543	0.3372092	
120 mg/ml	60 mg/ml	0.1090000	-0.075876	0.2938759	
100 mg/ml	60 mg/ml	0.0933333	-0.091543	0.2782092	
60 mg/ml	Media	0.0913333	-0.170121	0.3527873	
60 mg/ml	TCP	0.0853333	-0.099543	0.2702092	
140 mg/ml	60 mg/ml	0.0670000	-0.117876	0.2518759	
120 mg/ml	140 mg/ml	0.0420000	-0.142876	0.2268759	
100 mg/ml	140 mg/ml	0.0263333	-0.158543	0.2112092	
120 mg/ml	100 mg/ml	0.0156667	-0.169209	0.2005426	
TCP	Media	0.0060000	-0.255454	0.2674540	

www.manaraa.com

Oneway Analysis of Day 21 by PDO scaffolds of varying concentration for aFGF secretion



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	140 mg/ml	80 mg/ml	120 mg/ml	60 mg/ml	100 mg/ml	TCP	Media
140 mg/ml	-0.17096	-0.15763	-0.13596	-0.04663	0.01804	0.04104	0.06522
80 mg/ml	-0.15763	-0.17096	-0.14930	-0.05996	0.00470	0.02770	0.05189
120 mg/ml	-0.13596	-0.14930	-0.17096	-0.08163	-0.01696	0.00604	0.03022
60 mg/ml	-0.04663	-0.05996	-0.08163	-0.17096	-0.10630	-0.08330	-0.05911
100 mg/ml	0.01804	0.00470	-0.01696	-0.10630	-0.17096	-0.14796	-0.12378
TCP	0.04104	0.02770	0.00604	-0.08330	-0.14796	-0.17096	-0.14678
Media	0.06522	0.05189	0.03022	-0.05911	-0.12378	-0.14678	-0.29612

Positive values show pairs of means that are significantly different.

Level				Mean
140 mg/ml	A			0.43100000
80 mg/ml	A			0.41766667
120 mg/ml	A	B		0.39600000
60 mg/ml	A	B	C	0.30666667
100 mg/ml		B	C	0.24200000
TCP			C	0.21900000
Media			C	0.12400000

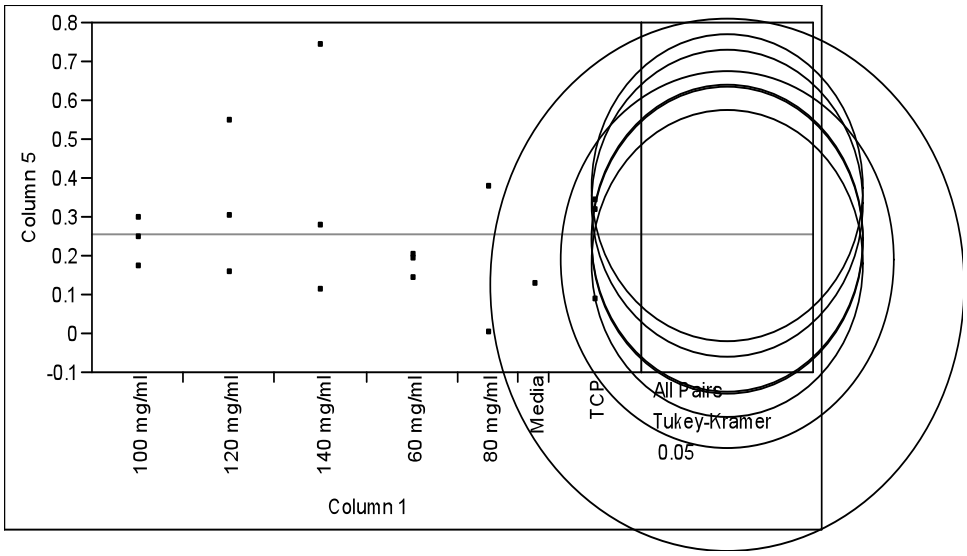
Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
140 mg/ml	Media	0.3070000	0.065221	0.5487786	
80 mg/ml	Media	0.2936667	0.051888	0.5354453	
120 mg/ml	Media	0.2720000	0.030221	0.5137786	
140 mg/ml	TCP	0.2120000	0.041037	0.3829633	
80 mg/ml	TCP	0.1986667	0.027703	0.3696300	
140 mg/ml	100 mg/ml	0.1890000	0.018037	0.3599633	
60 mg/ml	Media	0.1826667	-0.059112	0.4244453	
120 mg/ml	TCP	0.1770000	0.006037	0.3479633	
80 mg/ml	100 mg/ml	0.1756667	0.004703	0.3466300	

www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
120 mg/ml	100 mg/ml	0.1540000	-0.016963	0.3249633	
140 mg/ml	60 mg/ml	0.1243333	-0.046630	0.2952966	
100 mg/ml	Media	0.1180000	-0.123779	0.3597786	
80 mg/ml	60 mg/ml	0.1110000	-0.059963	0.2819633	
TCP	Media	0.0950000	-0.146779	0.3367786	
120 mg/ml	60 mg/ml	0.0893333	-0.081630	0.2602966	
60 mg/ml	TCP	0.0876667	-0.083297	0.2586300	
60 mg/ml	100 mg/ml	0.0646667	-0.106297	0.2356300	
140 mg/ml	120 mg/ml	0.0350000	-0.135963	0.2059633	
100 mg/ml	TCP	0.0230000	-0.147963	0.1939633	
80 mg/ml	120 mg/ml	0.0216667	-0.149297	0.1926300	
140 mg/ml	80 mg/ml	0.0133333	-0.157630	0.1842966	

Oneway Analysis of Day 28 by PDO scaffolds of varying concentration for aFGF secretion



Missing Rows
1

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.55539	0.05

Abs(Dif)-LSD	140 mg/ml	120 mg/ml	TCP	100 mg/ml	80 mg/ml	60 mg/ml	Media
140 mg/ml	-0.56167	-0.52034	-0.43200	-0.42300	-0.44146	-0.36300	-0.54332
120 mg/ml	-0.52034	-0.56167	-0.47334	-0.46434	-0.48280	-0.40434	-0.58465
TCP	-0.43200	-0.47334	-0.56167	-0.55267	-0.57113	-0.49267	-0.67299
100 mg/ml	-0.42300	-0.46434	-0.55267	-0.56167	-0.58013	-0.50167	-0.68199
80 mg/ml	-0.44146	-0.48280	-0.57113	-0.58013	-0.68790	-0.61580	-0.77800
60 mg/ml	-0.36300	-0.40434	-0.49267	-0.50167	-0.61580	-0.56167	-0.74199
Media	-0.54332	-0.58465	-0.67299	-0.68199	-0.77800	-0.74199	-0.97284

Positive values show pairs of means that are significantly different.

Level		Mean
140 mg/ml	A	0.37500000
120 mg/ml	A	0.33366667
TCP	A	0.24533333
100 mg/ml	A	0.23633333

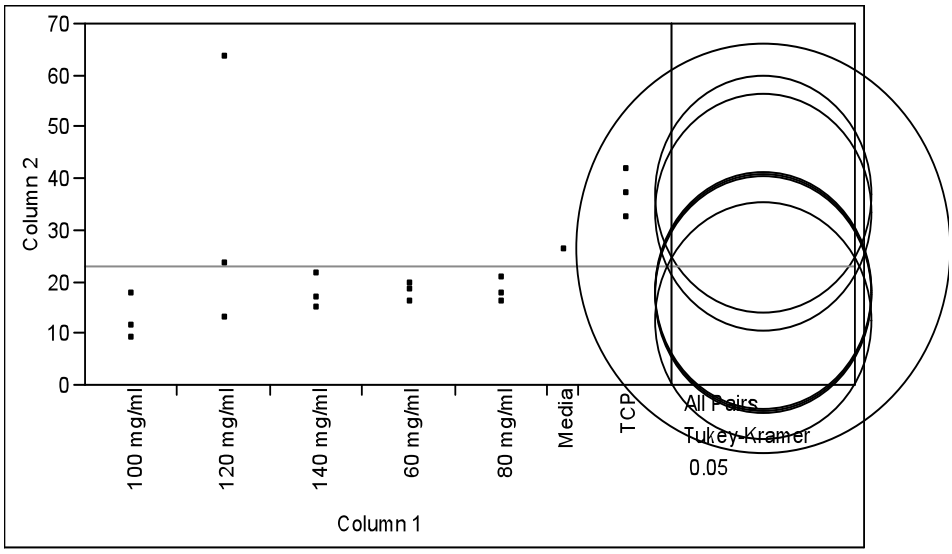
www.manaraa.com

Level		Mean
80 mg/ml	A	0.18850000
60 mg/ml	A	0.17633333
Media	A	0.12400000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
140 mg/ml	Media	0.2510000	-0.543320	1.045320	
120 mg/ml	Media	0.2096667	-0.584653	1.003987	
140 mg/ml	60 mg/ml	0.1986667	-0.363002	0.760336	
140 mg/ml	80 mg/ml	0.1865000	-0.441465	0.814465	
120 mg/ml	60 mg/ml	0.1573333	-0.404336	0.719002	
120 mg/ml	80 mg/ml	0.1451667	-0.482798	0.773132	
140 mg/ml	100 mg/ml	0.1386667	-0.423002	0.700336	
140 mg/ml	TCP	0.1296667	-0.432002	0.691336	
TCP	Media	0.1213333	-0.672987	0.915653	
100 mg/ml	Media	0.1123333	-0.681987	0.906653	
120 mg/ml	100 mg/ml	0.0973333	-0.464336	0.659002	
120 mg/ml	TCP	0.0883333	-0.473336	0.650002	
TCP	60 mg/ml	0.0690000	-0.492669	0.630669	
80 mg/ml	Media	0.0645000	-0.778003	0.907003	
100 mg/ml	60 mg/ml	0.0600000	-0.501669	0.621669	
TCP	80 mg/ml	0.0568333	-0.571132	0.684798	
60 mg/ml	Media	0.0523333	-0.741987	0.846653	
100 mg/ml	80 mg/ml	0.0478333	-0.580132	0.675798	
140 mg/ml	120 mg/ml	0.0413333	-0.520336	0.603002	
80 mg/ml	60 mg/ml	0.0121667	-0.615798	0.640132	
TCP	100 mg/ml	0.0090000	-0.552669	0.570669	

Fit Y by X Group
Oneway Analysis of Day 7 By PDO scaffolds of varying concentration for TGF-β1 secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha

www.manaraa.com

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	TCP	120 mg/ml	Media	80 mg/ml	60 mg/ml	140 mg/ml	100 mg/ml
TCP	-32.505	-28.787	-35.204	-13.747	-13.405	-13.104	-8.029
120 mg/ml	-28.787	-32.505	-38.922	-17.465	-17.123	-16.822	-11.747
Media	-35.204	-38.922	-56.301	-37.977	-37.635	-37.333	-32.258
80 mg/ml	-13.747	-17.465	-37.977	-32.505	-32.163	-31.862	-26.787
60 mg/ml	-13.405	-17.123	-37.635	-32.163	-32.505	-32.204	-27.129
140 mg/ml	-13.104	-16.822	-37.333	-31.862	-32.204	-32.505	-27.430
100 mg/ml	-8.029	-11.747	-32.258	-26.787	-27.129	-27.430	-32.505

Positive values show pairs of means that are significantly different.

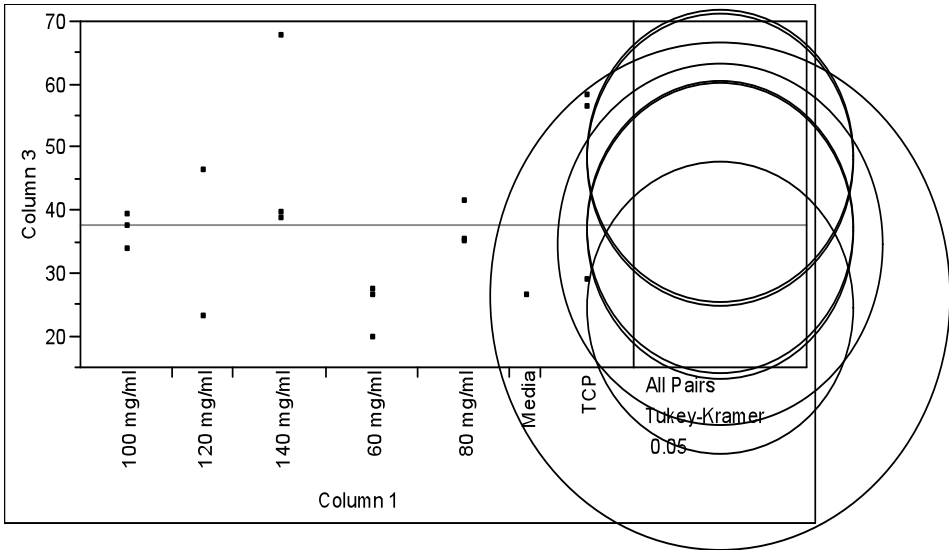
Level		Mean
TCP	A	36.930333
120 mg/ml	A	33.212000
Media	A	26.165000
80 mg/ml	A	18.172333
60 mg/ml	A	17.830333
140 mg/ml	A	17.529000
100 mg/ml	A	12.454000

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
TCP	100 mg/ml	24.47633	-8.0288	56.98144	
120 mg/ml	100 mg/ml	20.75800	-11.7471	53.26311	
TCP	140 mg/ml	19.40133	-13.1038	51.90644	
TCP	60 mg/ml	19.10000	-13.4051	51.60511	
TCP	80 mg/ml	18.75800	-13.7471	51.26311	
120 mg/ml	140 mg/ml	15.68300	-16.8221	48.18811	
120 mg/ml	60 mg/ml	15.38167	-17.1234	47.88678	
120 mg/ml	80 mg/ml	15.03967	-17.4654	47.54478	
Media	100 mg/ml	13.71100	-32.2582	59.68017	
TCP	Media	10.76533	-35.2038	56.73450	
Media	140 mg/ml	8.63600	-37.3332	54.60517	
Media	60 mg/ml	8.33467	-37.6345	54.30384	
Media	80 mg/ml	7.99267	-37.9765	53.96184	
120 mg/ml	Media	7.04700	-38.9222	53.01617	
80 mg/ml	100 mg/ml	5.71833	-26.7868	38.22344	
60 mg/ml	100 mg/ml	5.37633	-27.1288	37.88144	
140 mg/ml	100 mg/ml	5.07500	-27.4301	37.58011	
TCP	120 mg/ml	3.71833	-28.7868	36.22344	
80 mg/ml	140 mg/ml	0.64333	-31.8618	33.14844	
80 mg/ml	60 mg/ml	0.34200	-32.1631	32.84711	
60 mg/ml	140 mg/ml	0.30133	-32.2038	32.80644	

www.manaraa.com

Oneway Analysis of Day 14 By PDO scaffolds of varying concentration for TGF-β1 secretion



Missing Rows
1

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.55539	0.05

Abs(Dif)-LSD	140 mg/ml	TCP	80 mg/ml	100 mg/ml	120 mg/ml	Media	60 mg/ml
140 mg/ml	-33.133	-32.351	-21.775	-21.320	-22.955	-24.467	-8.921
TCP	-32.351	-33.133	-22.557	-22.102	-23.737	-25.249	-9.702
80 mg/ml	-21.775	-22.557	-33.133	-32.678	-34.312	-35.824	-20.278
100 mg/ml	-21.320	-22.102	-32.678	-33.133	-34.767	-36.279	-20.733
120 mg/ml	-22.955	-23.737	-34.312	-34.767	-40.579	-41.398	-26.920
Media	-24.467	-25.249	-35.824	-36.279	-41.398	-57.388	-45.034
60 mg/ml	-8.921	-9.702	-20.278	-20.733	-26.920	-45.034	-33.133

Positive values show pairs of means that are significantly different.

Level		Mean
140 mg/ml	A	48.554667
TCP	A	47.773000
80 mg/ml	A	37.197333
100 mg/ml	A	36.742333
120 mg/ml	A	34.466000
Media	A	26.165000
60 mg/ml	A	24.342667

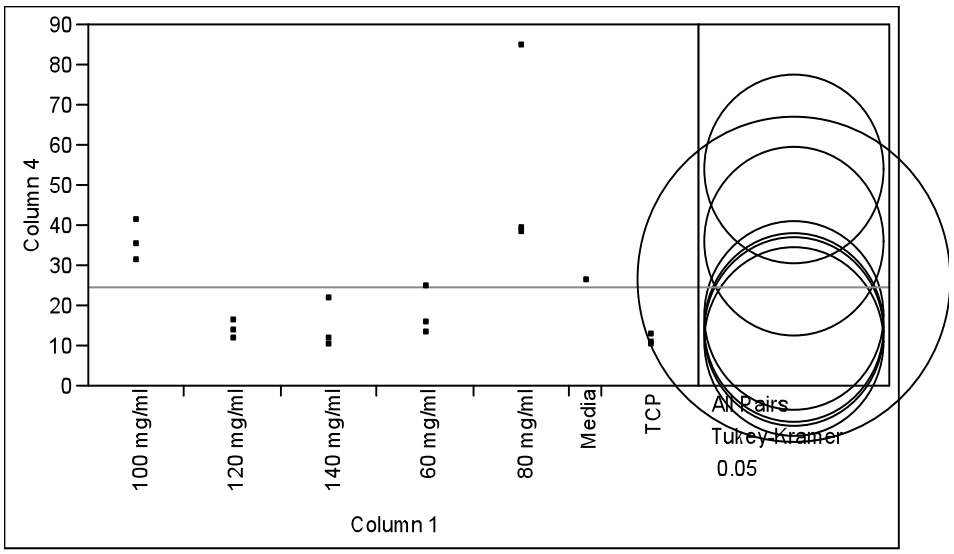
Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
140 mg/ml	60 mg/ml	24.21200	-8.9207	57.34473	
TCP	60 mg/ml	23.43033	-9.7024	56.56306	
140 mg/ml	Media	22.38967	-24.4671	69.24642	
TCP	Media	21.60800	-25.2487	68.46475	
140 mg/ml	120 mg/ml	14.08867	-22.9548	51.13218	
TCP	120 mg/ml	13.30700	-23.7365	50.35051	
80 mg/ml	60 mg/ml	12.85467	-20.2781	45.98739	

www.manaraa.com

Level	- Level	Difference	Lower CL	Upper CL	Difference
100 mg/ml	60 mg/ml	12.39967	-20.7331	45.53239	
140 mg/ml	100 mg/ml	11.81233	-21.3204	44.94506	
140 mg/ml	80 mg/ml	11.35733	-21.7754	44.49006	
80 mg/ml	Media	11.03233	-35.8244	57.88908	
TCP	100 mg/ml	11.03067	-22.1021	44.16339	
100 mg/ml	Media	10.57733	-36.2794	57.43408	
TCP	80 mg/ml	10.57567	-22.5571	43.70839	
120 mg/ml	60 mg/ml	10.12333	-26.9202	47.16685	
120 mg/ml	Media	8.30100	-41.3981	58.00009	
80 mg/ml	120 mg/ml	2.73133	-34.3122	39.77485	
100 mg/ml	120 mg/ml	2.27633	-34.7672	39.31985	
Media	60 mg/ml	1.82233	-45.0344	48.67908	
140 mg/ml	TCP	0.78167	-32.3511	33.91439	
80 mg/ml	100 mg/ml	0.45500	-32.6777	33.58773	

Oneway Analysis of Day 21 By PDO scaffolds of varying concentration for TGF-β1 secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

Abs(Dif)-LSD	80 mg/ml	100 mg/ml	Media	60 mg/ml	140 mg/ml	120 mg/ml	TCP
80 mg/ml	-33.333	-15.219	-19.427	2.962	6.130	7.105	9.451
100 mg/ml	-15.219	-33.333	-37.540	-15.152	-11.983	-11.008	-8.663
Media	-19.427	-37.540	-57.734	-38.558	-35.390	-34.415	-32.069
60 mg/ml	2.962	-15.152	-38.558	-33.333	-30.164	-29.189	-26.844
140 mg/ml	6.130	-11.983	-35.390	-30.164	-33.333	-32.358	-30.012
120 mg/ml	7.105	-11.008	-34.415	-29.189	-32.358	-33.333	-30.987
TCP	9.451	-8.663	-32.069	-26.844	-30.012	-30.987	-33.333

Positive values show pairs of means that are significantly different.

Level			Mean
80 mg/ml	A		53.878000
100 mg/ml	A	B	35.764667
Media	A	B	26.165000

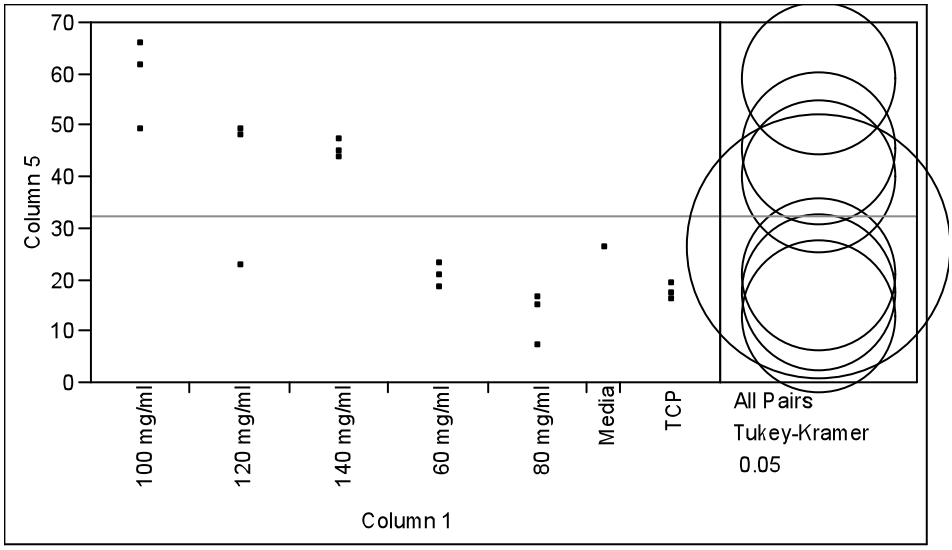
www.manaraa.com

Level		Mean
60 mg/ml	B	17.583667
140 mg/ml	B	14.415000
120 mg/ml	B	13.440000
TCP	B	11.094667

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
80 mg/ml	TCP	42.78333	9.4506	76.11611	
80 mg/ml	120 mg/ml	40.43800	7.1052	73.77078	
80 mg/ml	140 mg/ml	39.46300	6.1302	72.79578	
80 mg/ml	60 mg/ml	36.29433	2.9616	69.62711	
80 mg/ml	Media	27.71300	-19.4267	74.85267	
100 mg/ml	TCP	24.67000	-8.6628	58.00278	
100 mg/ml	120 mg/ml	22.32467	-11.0081	55.65744	
100 mg/ml	140 mg/ml	21.34967	-11.9831	54.68244	
100 mg/ml	60 mg/ml	18.18100	-15.1518	51.51378	
80 mg/ml	100 mg/ml	18.11333	-15.2194	51.44611	
Media	TCP	15.07033	-32.0693	62.21000	
Media	120 mg/ml	12.72500	-34.4147	59.86467	
Media	140 mg/ml	11.75000	-35.3897	58.88967	
100 mg/ml	Media	9.59967	-37.5400	56.73933	
Media	60 mg/ml	8.58133	-38.5583	55.72100	
60 mg/ml	TCP	6.48900	-26.8438	39.82178	
60 mg/ml	120 mg/ml	4.14367	-29.1891	37.47644	
140 mg/ml	TCP	3.32033	-30.0124	36.65311	
60 mg/ml	140 mg/ml	3.16867	-30.1641	36.50144	
120 mg/ml	TCP	2.34533	-30.9874	35.67811	
140 mg/ml	120 mg/ml	0.97500	-32.3578	34.30778	

Oneway Analysis of Day 28 By PDO scaffolds of varying concentration for TGF-β1 secretion



Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
3.49978	0.05

www.manaraa.com

Abs(Dif)-LSD	100 mg/ml	140 mg/ml	120 mg/ml	Media	60 mg/ml	TCP	80 mg/ml
100 mg/ml	-21.200	-7.652	-2.310	2.596	16.889	20.056	24.735
140 mg/ml	-7.652	-21.200	-15.859	-10.952	3.340	6.507	11.187
120 mg/ml	-2.310	-15.859	-21.200	-16.294	-2.001	1.166	5.845
Media	2.596	-10.952	-16.294	-36.720	-24.471	-21.304	-16.625
60 mg/ml	16.889	3.340	-2.001	-24.471	-21.200	-18.033	-13.354
TCP	20.056	6.507	1.166	-21.304	-18.033	-21.200	-16.521
80 mg/ml	24.735	11.187	5.845	-16.625	-13.354	-16.521	-21.200

Positive values show pairs of means that are significantly different.

Level					Mean
100 mg/ml	A				58.743333
140 mg/ml	A	B			45.194667
120 mg/ml	A	B	C		39.853333
Media		B	C	D	26.165000
60 mg/ml			C	D	20.654000
TCP				D	17.487000
80 mg/ml				D	12.807667

Levels not connected by same letter are significantly different.

Level	- Level	Difference	Lower CL	Upper CL	Difference
100 mg/ml	80 mg/ml	45.93567	24.7352	67.13612	
100 mg/ml	TCP	41.25633	20.0559	62.45679	
100 mg/ml	60 mg/ml	38.08933	16.8889	59.28979	
100 mg/ml	Media	32.57833	2.5964	62.56030	
140 mg/ml	80 mg/ml	32.38700	11.1865	53.58745	
140 mg/ml	TCP	27.70767	6.5072	48.90812	
120 mg/ml	80 mg/ml	27.04567	5.8452	48.24612	
140 mg/ml	60 mg/ml	24.54067	3.3402	45.74112	
120 mg/ml	TCP	22.36633	1.1659	43.56679	
120 mg/ml	60 mg/ml	19.19933	-2.0011	40.39979	
140 mg/ml	Media	19.02967	-10.9523	49.01164	
100 mg/ml	120 mg/ml	18.89000	-2.3105	40.09045	
120 mg/ml	Media	13.68833	-16.2936	43.67030	
100 mg/ml	140 mg/ml	13.54867	-7.6518	34.74912	
Media	80 mg/ml	13.35733	-16.6246	43.33930	
Media	TCP	8.67800	-21.3040	38.65997	
60 mg/ml	80 mg/ml	7.84633	-13.3541	29.04679	
Media	60 mg/ml	5.51100	-24.4710	35.49297	
140 mg/ml	120 mg/ml	5.34133	-15.8591	26.54179	
TCP	80 mg/ml	4.67933	-16.5211	25.87979	
60 mg/ml	TCP	3.16700	-18.0335	24.36745	

www.manaraa.com

VITA

Koyal Garg was born in Meerut, U.P., India on 29th September 1985. She received a Bachelors of Engineering degree in Biomedical Instrumentation Engineering from Avinashilingam Deemed University, India in May 2006. In fall of 2006, she started graduate studies in Virginia Commonwealth University in Biomedical Engineering. She is a member of the Virginia Commonwealth University chapter of the Alpha Eta Mu Beta Biomedical Engineering Honor Society and the Biomedical Engineering Society (BMES). She has presented posters at various conferences including Annual Hilton Head

Workshop, BMES annual fall meeting and The International Society for Applied Cardiovascular Biology (ISACB) Biennial meeting. She had also worked as a Teaching Assistant for several courses, such as Biomedical Instrumentation, Computational Methods and Biomedical Signal Processing.

www.manaraa.com