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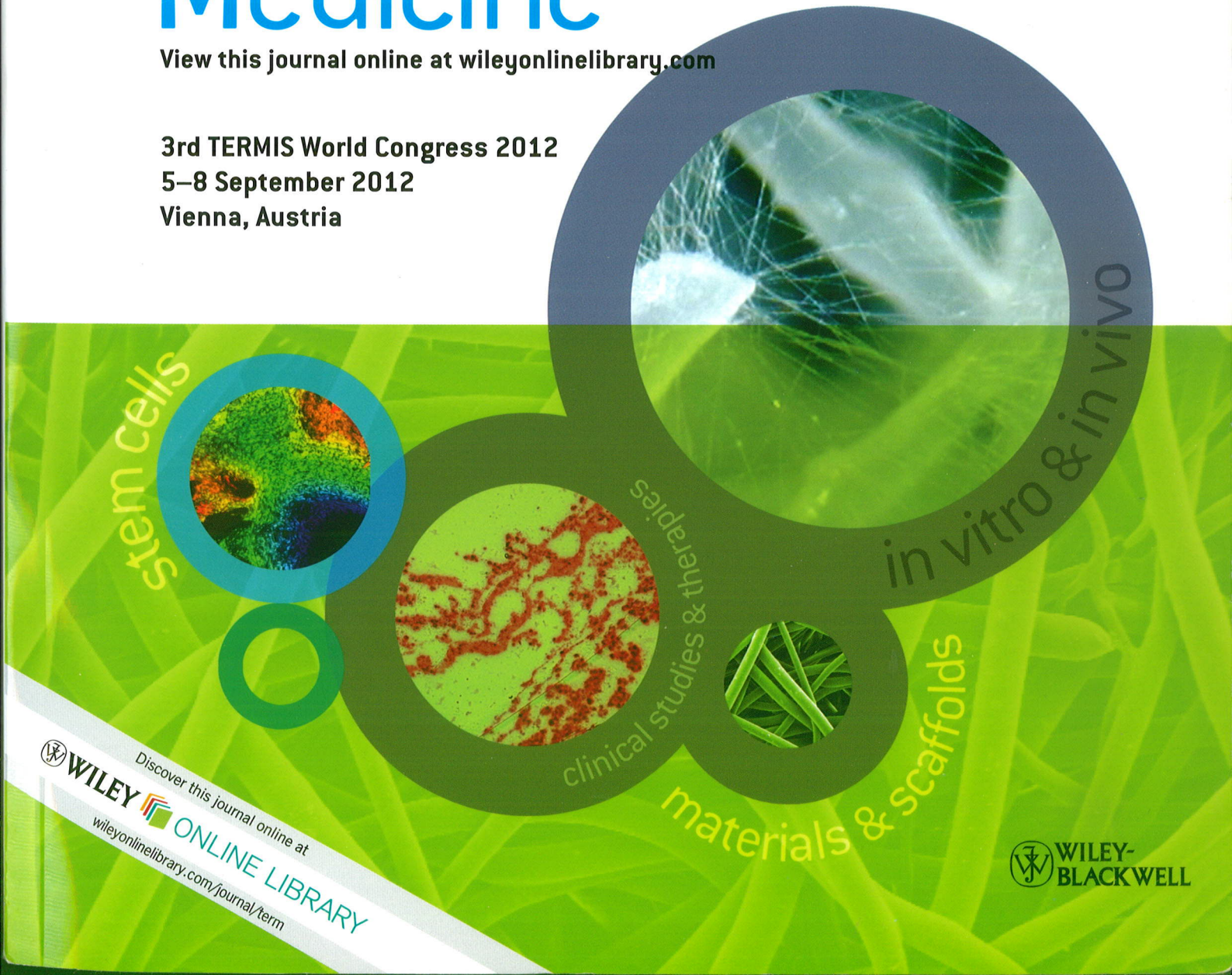

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61. 3D Scaffold/Cell Printing

61.01
Bioprinting anisotropic stem cell microenvironment

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Current cell/tissue scaffolding methods present challenges due to lack of control over spatial cell seeding and extracellular matrix (ECM) composition. Microdroplet-based hydrogel bioprinting can be used to engineer complex tissue anisotropies by producing scaffolds with controlled microscale spatial heterogeneity in ECM and cellular compositions. We showed that microdroplet-based hydrogel bioprinting approach will facilitate engineering of complex tissue anisotropies. We achieved a biochemical gradient with microdroplets encapsulating human Mesenchymal Stem Cells (hMSCs) and to assess the hMSC differentiation at the engineered fibrocartilage phase. Microdroplets were created by cell-encapsulating droplet generation system and cell-laden collagen droplets were printed on collagen coated substrate. hMSCs were patterned in microdroplets with BMP-2 and TGF-β1 representing the fibrocartilage phase. Quantitative RT-PCR array was used to assess the differentiation of hMSCs to bone and cartilage following 16 days of culture. PCR analysis showed that the hMSCs displayed a potent upregulation of osteogenesis and chondrogenesis related genes. MSC related genes as well as other tissue specific (e.g., adipose and tendon) genes were down regulated at the micropatterned interface. These results indicated that microdroplet-based bioprinting can generate biochemical anisotropy to facilitate controlled hMSC differentiation in bone-fibrocartilage phase.

61.02
Development and validation of an integrated organ printing system

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Although a variety of tissue constructs can be generated with gel materials, these often present with limitations in maintaining structural integrity due to their low mechanical strength, which makes handling and surgical suturing difficult. As such, we developed a novel organ printing system that integrates solid freeform fabrication and cell printing technology. The integrated organ printing system consisted of four delivery modules that can process multiple types of materials, including synthetic polymers and a cell/gel mixture. The materials are precisely dispensed by the control of air pressure. A heating unit was used to obtain dispensable synthetic polymers. We investigated the feasibility of delivering viable cells while fabricating rigid synthetic scaffolds simultaneously to create durable tissue structures. NIH 3T3 cells/Pluronic F-127 mixture, gelatin, and poly(ϵ-caprolactone) (PCL) were delivered to construct 3D structures. Live/dead staining and MTS assays were performed to evaluate cell viability within the 3D configuration. The results show that the cells were successfully placed into the desired position with approximately 80% cell viability. We have successfully designed and constructed an integrated organ printing system that is able to print 3D-organ structures with precision. This system provides a major leap in the advancement of organ printing technology.

61.03
Monitoring analyte concentration using self reporting scaffolds a 3D culture model

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Within a 3D cell culture construct it is favourable to have a homogenous cell culture environment that is free from analyte gradients such as those created from inefficient nutrient exchange or metabolic waste removal. Assessment of analyte concentration within a cultured 3D environment can be performed by physically probing the construct; however this can disturb cell viability and function and ultimately led to sacrifice of the tissue model. We have developed novel self-reporting scaffolds that can be utilised as an analytical tool to monitor local environmental analyte concentrations within 3D cell culture models. The self-reporting scaffolds incorporate optically responsive nanosensors within the polymer fibres to produce scaffolds capable of non-invasive *in situ* assessment of local environmental analyte concentrations such as oxygen and pH. The nanosensors report the analyte concentration by ratiometric fluorescent output from dyes incorporated into a biocompatible matrix. Culture models positioned within a bioreactor can prevent 3D culture-associated cell death but may not entirely eliminate analyte gradients. Coupling the use of self-reporting scaffolds and perfusion bioreactors can in the future provide on-line assessment of dynamic 3D culture.

61.04
Laser fabrication of multi-scale elastic 3D scaffolds

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Until recently 3D cell-culture matrices were mainly considered from a standpoint of support and guidance of cell proliferation and tissue development. The early designs of tissue engineering constructs focused on bulk properties, while disregarding individual cell environment. Current findings show that the role of the extracellular matrix (ECM) extends beyond a simple structural support to regulation of cell and tissue function. Mechanical stimulation and cell interaction with the topographic cues is an important part of this regulation. Systematic studies of its underlying mechanisms *in vitro* rely on methods capable of creating such 3D cell-culture matrices with high reproducibility and in accordance to a defined design. Two-photon polymerization (2PP) is a method based on localized cross-linking of the photopolymers, induced by femtosecond laser pulses. High resolution of the 2PP facilitates fabrication of 3D scaffolds, which contain features at several length scales, in a single step. In this contribution our recent results on fabrication of 3D scaffolds by 2PP of novel biodegradable photoelastomers are presented. The scaffolds were seeded with different cell types, and cultured under mechanical stimuli and in static conditions. Our results emphasize the potential of the proposed method for realization of rationally engineered multi-scale 3D scaffolds.

61.P07
Developing biodegradable 3D hydrogels scaffolds: from synthesis to two-photon polymerization

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Developing 3D hydrogel scaffolds capable of promoting cell viability and cell-extracellular matrix (ECM) interactions is of great importance for Tissue Engineering (TE) applications. To mimic the biochemical and structural complexity of ECM, two-photon polymerization (TPP) shows the greatest promise to fabricate ECM-biomimetic hydrogels because it allows fabrication of complex user-dictated shapes with micrometer-scale resolution. Poly(ethylene glycol) diacrylates have been widely used for TPP fabrication of hydrogels due to high reactivity. However, unreacted acrylate groups are irritant and potentially cytotoxic. For most TE applications, monomers with low cyto-toxicity are in demand. Our previous work proved that vinyl esters (VEs) are much less cytotoxic than acrylates references. Although VEs are generally not as reactive as acrylates, the thiol-vinyl ester photo-click reactions are robust enough for efficient TPP applications. Since it is hard to assemble ECM-biomimetic hydrogels using synthetic monomers alone, proteins with Thiol and VE functionalities are developed for their potential use as Thiol-ene photo-click hydrogels. Presented is the synthesis of vinyl ester derivative of gelatin hydrolysate, preparation of thiolated bovine serum albumin, and 3D fabrication of gelatin/albumin based hydrogel scaffolds via TPP click reactions. We suppose the robust thiol-vinyl ester photo-click reactions are responsible for the reasonable writing speed and broad processing window.

61.P08
Water soluble initiators for two-photon polymerization

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Two-photon polymerization (2PP) in aqueous environment has attracted increasing attentions due to its versatility in biological applications, especially in the microfabrication of biocompatible 3D scaffolds for tissue engineering with nano-level resolution. Efficient water-borne two-photon curable formulation demands reactive water-soluble photoinitiators (PIs) with low toxicities. Although some commercial hydrophilic dyes have been used as initiators in 3D biological fabrication, high laser intensities and long exposure time are required due to their small two-photon absorption (TPA) cross sections. Here, a novel water-soluble TPA PI 2, 5-bis-[4-[(2-sodiumcarboxylate-methyl)(methyl)-amino]-benzylidene]-4- methylcyclohexanone (G2CK) was prepared via introducing hydrophilic sodium carboxylate groups into the efficient TPA PI 2,5-bis-[4-(dimthylamino)-benzylidene]-4-methylcyclohexanone, which showed large TPA cross section combined with high photoinitiation activity. In 2PP structuring tests using formulations with water contents of up to 80%, G2CK exhibited broad ideal processing windows and nicely shaped structures were obtained at low laser intensities, as well as high writing speeds. Finally, the results of time resolved cytotoxicity tests with this novel water-soluble PI are presented.

61.P09
Controllable and tunable 3D scaffolds fabrication using laser based techniques

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In tissue engineering, the development of a reproducible and controlled method of micro/nano fabrication of 3D scaffolds is of great importance. Ultrafast pulsed lasers micro/nano engineering offers unique features; nanoscale spatial resolution, nonthermal and nondestructive engineering. Here, we report on the fabrication of high resolution 3D scaffolds deploying three laser based techniques: (i) two-photon polymerization (2PP), (ii) ultrafast laser micro/nano structuring (ULMNS) (iii) single pulse UV laser irradiation of biopolymers. The 2PP technique relies on two-photon polymerization enabling 3D bioactive structures fabrication with submicron resolution. The ULMNS technique is an effective direct-write method to fabricate hierarchical micro/nano structures on Si surfaces with superior control over structure geometry and pattern regularity. We report Si micro/nano spike forests fabrication and replication of the initial spiked morphology on soft polymeric materials. The third technique relies on the generation of a foam layer on biopolymers. Following scaffolds' fabrication, tailoring of their surface properties has been advantageously achieved. The ability of different cell types to be grown and differentiated onto the specific scaffolds has been investigated. The ultimate goal is to explore the influence of the synergy of surface topography and chemistry on cell behaviour, related to viability, motility, adhesion, morphology, cytoskeletal arrangement and differentiation.

61.P10
Application of riboflavin as water-soluble photoinitiator for fabrication of tissue engineering scaffolds via two-photon polymerization

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In recent years, two-photon polymerization (2PP) became a powerful tool for fabrication of different biomedical micro-devices ranging from microneedles for transdermal drug delivery and ossicular prosthesis for middle ear to scaffolds for tissue engineering (TE). Applying 2PP, precisely defined complex 3D scaffolds for TE can be fabricated from variety of materials such as completely synthetic and non-biodegradable acrylates, PEG-hydrogels and modified natural proteins. However, cytotoxity of the photoinitiators is the factor which limits the use of 2PP technology for fabrication of TE scaffolds. One potential source of biocompatible photoinitiators is free radical generating molecules that are naturally occurring in biological systems. Riboflavin (vitamin B2) is one chemical that has potential to be used as a more biocompatible photoinitiator. In addition to a biocompatible photoinitiator, a biocompatible polymer that can be cross-linked by free radicals is needed for making tissue engineering scaffolds by 2PP. We have demonstrated, for the first time, the ability to perform two-photon polymerization using riboflavin as a photoinitiator. TE scaffolds produced out of PEGda with riboflavin as a photoinitiator were found to support cell growth. Toxicity testing indicated that PEGda crosslinked using riboflavin has superior biocompatibility, much greater than when crosslinked using conventional photoinitiators, such as Irgacure 369 and Irgacure 2959.