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Literature List (June 2015)

Listed below are research papers published by our customers using our 3D-Bioplotter technology. This is not a complete list and no preference was applied in ordering the individual papers. If you find a paper not included, or wish to exclude a paper of which you are an author, please contact us.

2000

Landers, Rüdiger, and Rolf Mülhaupt. "**Desktop manufacturing of complex objects, prototypes and biomedical scaffolds by means of computer-assisted design combined with computer-guided 3D plotting of polymers and reactive oligomers.**" *Macromolecular Materials and Engineering* 282.1 (2000): 17-21.

Computer-assisted design and image processing were combined with computer-guided one- and two-component air-driven 3D dispensing of hotmelts, solutions, pastes, dispersions of polymers as well as monomers and reactive oligomers to produce solid objects with complex shapes and tailor-made internal structures. During the 3D plotting process either individual microdots or microstrands were positioned in order to construct complex objects, fibers, tubes and scaffolds similar to non-wovens. The resolution was in the range of 200 µm and depended upon inner nozzle diameter, air pressure, plotting speed, rheology, and plotting medium. Plotting in liquid media with densities similar to that of the dispensing liquid eliminated the need for construction of temporary support structures. The design capabilities of this computer-guided 3D plotting process was demonstrated using conventional moisture-curable silicone resin.

2002

Landers, R., et al. "**Fabrication of soft tissue engineering scaffolds by means of rapid prototyping techniques.**" *Journal of Materials Science* 37.15 (2002): 3107-3116.

Scaffolds are of great importance for tissue engineering because they enable the production of functional living implants out of cells obtained from cell culture. These scaffolds require individual external shape and well defined internal structure with interconnected porosity. The problem of the fabrication of prototypes from computer assisted design (CAD) data is well known in automotive industry. Rapid prototyping (RP) techniques are able to produce such parts. Some RP techniques exist for hard tissue implants. Soft tissue scaffolds need a hydrogel material. No biofunctional and cell compatible processing for hydrogels exists in the area of RP. Therefore, a new rapid prototyping (RP) technology was developed at the Freiburg Materials Research Center to meet the demands for desktop fabrication of hydrogels. A key feature of this RP technology is the three-dimensional dispensing of liquids and pastes in liquid media. The porosity of the scaffold is calculated and an example of the data conversion from a volume model to the plotting path control is demonstrated. The versatile applications of the new hydrogel scaffolds are discussed, including especially its potential for tissue engineering.

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Landers, Rüdiger, et al. "**Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering.**" *Biomaterials* 23.23 (2002): 4437-4447.

In the year 2000 a new rapid prototyping (RP) technology was developed at the Freiburg Materials Research Center to meet the demands for desktop fabrication of scaffolds useful in tissue engineering. A key feature of this RP technology is the three-dimensional (3D) dispensing of liquids and pastes in liquid media. In contrast to conventional RP systems, mainly focused on melt processing, the 3D dispensing RP process (3D plotting) can apply a much larger variety of synthetic as well as natural materials, including aqueous solutions and pastes, to fabricate scaffolds for application in tissue engineering. For the first time, hydrogel scaffolds with a designed external shape and a well-defined internal pore structure were prepared by this RP process. Surface coating and pore formation were achieved to facilitate cell adhesion and cell growth. The versatile application potential of new hydrogel scaffolds was demonstrated in cell culture.

2004

Pfister, Andreas, et al. "**Biofunctional rapid prototyping for tissue-engineering applications: 3D bioplotting versus 3D printing.**" *Journal of Polymer Science Part A: Polymer Chemistry* 42.3 (2004): 624-638.

Two important rapid-prototyping technologies (3D Printing and 3D Bioplotting) were compared with respect to the computer-aided design and free-form fabrication of biodegradable polyurethane scaffolds meeting the demands of tissue-engineering applications. Aliphatic polyurethanes were based on lysine ethyl ester diisocyanate and isophorone diisocyanate. Layer-by-layer construction of the scaffolds was performed by 3D Printing, that is, bonding together starch particles followed by infiltration and partial crosslinking of starch with lysine ethyl ester diisocyanate. Alternatively, the 3D Bioplotting process permitted three-dimensional dispensing and reactive processing of oligoetherurethanes derived from isophorone diisocyanate, oligoethylene oxide, and glycerol. The scaffolds were characterized with X-ray microtomography, scanning electron microscopy, and mechanical testing. Osteoblast-like cells were seeded on such scaffolds to demonstrate their potential in tissue engineering.

2005

Carvalho, C., et al. "**Fabrication of soft and hard biocompatible scaffolds using 3D-Bioplotting.**" *Virtual Modelling and Rapid Manufacturing-Advanced Research in Virtual and Rapid Prototyping*. London, England: Taylor & Francis Group (2005): 97-102.

In Tissue Engineering and bone reconstruction, alongside the choice of materials, the scaffold design is of great importance. Three dimensional structures not only permit the tuning of chemical and mechanical properties, but they can also copy the outer form of the required bone or cartilaginous structures. While new processes that create such 3D scaffolds by means of Rapid Prototyping have been developed, they are still restricted to a limited type of materials. At the Freiburger Materialforschungszentrum, our group has developed a new process called 3D BioplottingTM. Most kinds of polymers and biopolymers can be used for the fabrication of 3D scaffolds with 3D BioplottingTM, including hydrogels (e.g. collagen, agar), polymer melts (e.g. PLLA, PGA) and two-component systems (e.g. chitosan, fibrin). Tailor made biodegradable scaffolds can be fabricated in a short time using individual computer-tomography data from the patient. In-vitro tests showed promising results and in-vivo experiments are now under observation.

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Li, Jia Ping, et al. "**Porous Ti_6Al_4V scaffold directly fabricating by rapid prototyping: Preparation and in vitro experiment.**" *Biomaterials* 27.8 (2006): 1223-1235.

Three-dimensional (3D) fiber deposition (3DF), a rapid prototyping technology, was successfully directly applied to produce novel 3D porous Ti_6Al_4V scaffolds with fully interconnected porous networks and highly controllable porosity and pore size. A key feature of this technology is the 3D computer-controlled fiber depositing of Ti6Al4V slurry at room temperature to produce a scaffold, consisting of layers of directionally aligned Ti6Al4V fibers. In this study, the Ti6Al4V slurry was developed for the 3D fiber depositing process, and the parameters of 3D fiber depositing were optimized. The experimental results show how the parameters influence the structure of porous scaffold. The potential of this rapid prototyping 3DF system for fabricating 3D Ti6Al4V scaffolds with regular and reproducible architecture meeting the requirements of tissue engineering and orthopedic implants is demonstrated.

Moroni, Lorenzo, J. R. De Wijn, and C. A. Van Blitterswijk. "**3D fiber-deposited scaffolds for tissue engineering: influence of pores geometry and architecture on dynamic mechanical properties.**" *Biomaterials* 27.7 (2006): 974-985.

One of the main issues in tissue engineering is the fabrication of scaffolds that closely mimic the biomechanical properties of the tissues to be regenerated. Conventional fabrication techniques are not sufficiently suitable to control scaffold structure to modulate mechanical properties. Within novel scaffold fabrication processes 3D fiber deposition (3DF) showed great potential for tissue engineering applications because of the precision in making reproducible 3D scaffolds, characterized by 100% interconnected pores with different shapes and sizes. Evidently, these features also affect mechanical properties. Therefore, in this study we considered the influence of different structures on dynamic mechanical properties of 3DF scaffolds. Pores were varied in size and shape, by changing fibre diameter, spacing and orientation, and layer thickness. With increasing porosity, dynamic mechanical analysis (DMA) revealed a decrease in elastic properties such as dynamic stiffness and equilibrium modulus, and an increase of the viscous parameters like damping factor and creep unrecovered strain. Furthermore, the Poisson's ratio was measured, and the shear modulus computed from it. Scaffolds showed an adaptable degree of compressibility between sponges and incompressible materials. As comparison, bovine cartilage was tested and its properties fell in the fabricated scaffolds range. This investigation showed that viscoelastic properties of 3DF scaffolds could be modulated to accomplish mechanical requirements for tailored tissue engineered applications.

Moroni, Lorenzo, et al. "**Polymer hollow fiber three-dimensional matrices with controllable cavity and shell thickness.**" *Biomaterials* 27.35 (2006): 5918-5926.

Hollow fibers find useful applications in different disciplines like fluid transport and purification, optical guidance, and composite reinforcement. In tissue engineering, they can be used to direct tissue in-growth or to serve as drug delivery depots. The fabrication techniques currently available, however, do not allow to simultaneously organize them into three-dimensional (3D) matrices, thus adding further functionality to approach more complicated or hierarchical structures. We report here the development of a novel technology to fabricate hollow fibers with controllable hollow cavity diameter and shell thickness. By exploiting viscous encapsulation, a rheological phenomenon often undesired in molten polymeric blends flowing through narrow ducts, fibers with a shell–core configuration can be extruded. Hollow fibers are then obtained by selective dissolution of the inner core polymer. The hollow cavity diameter and the shell thickness can be controlled by varying the polymers in the blend, the blend composition, and the extrusion nozzle diameter. Simultaneous with extrusion, the extrudates are organized into 3D matrices with different architectures and custom-made shapes by 3D fiber deposition, a rapid prototyping tool which has recently been applied for the production of scaffolds for tissue engineering purposes. Applications in tissue engineering and controlled drug delivery of these constructs are presented and discussed.

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Moroni, L., et al. "**Dynamic mechanical properties of 3D fiber-deposited PEOT/PBT scaffolds: An experimental and numerical analysis.**" *Journal of Biomedical Materials Research Part A* 78.3 (2006): 605-614.

Mechanical properties of three-dimensional (3D) scaffolds can be appropriately modulated through novel fabrication techniques like 3D fiber deposition (3DF), by varying scaffold's pore size and shape. Dynamic stiffness, in particular, can be considered as an important property to optimize the scaffold structure for its ultimate in vivo application to regenerate a natural tissue. Experimental data from dynamic mechanical analysis (DMA) reveal a dependence of the dynamic stiffness of the scaffold on the intrinsic mechanical and physicochemical properties of the material used, and on the overall porosity and architecture of the construct. The aim of this study was to assess the relationship between the aforementioned parameters, through a mathematical model, which was derived from the experimental mechanical data. As an example of how mechanical properties can be tailored to match the natural tissue to be replaced, articular bovine cartilage and porcine knee meniscus cartilage dynamic stiffness were measured and related to the modeled 3DF scaffolds dynamic stiffness. The dynamic stiffness of 3DF scaffolds from poly(ethylene oxide terephthalate)–poly(butylene terephthalate) (PEOT/PBT) copolymers was measured with DMA. With increasing porosity, the dynamic stiffness was found to decrease in an exponential manner. The influence of the scaffold architecture (or pore shape) and of the molecular network properties of the copolymers was expressed as a scaffold characteristic coefficient α, which modulates the porosity effect. This model was validated through an FEA numerical simulation performed on the structures that were experimentally tested. The relative deviation between the experimental and the finite element model was less than 15% for all of the constructs with a dynamic stiffness higher than 1 MPa. Therefore, we conclude that the mathematical model introduced can be used to predict the dynamic stiffness of a porous PEOT/PBT scaffold, and to choose the biomechanically optimal structure for tissue engineering applications.

Rücker, Martin, et al. "**Angiogenic and inflammatory response to biodegradable scaffolds in dorsal skinfold chambers of mice.**" *Biomaterials* 27.29 (2006): 5027-5038.

For tissue engineering, scaffolds should be biocompatible and promote neovascularization. Because little is known on those specific properties, we herein studied in vivo the host angiogenic and inflammatory response after implantation of commonly used scaffold materials. Porous poly(l-lactide-co-glycolide) (PLGA) and collagen–chitosan–hydroxyapatite hydrogel scaffolds were implanted into dorsal skinfold chambers of balb/c mice. Additional animals received cortical bone as an isogeneic, biological implant, while chambers of animals without implants served as controls. Angiogenesis and neovascularization as well as leukocyte–endothelial cell interaction and microvascular permeability were analyzed over 14 day using intravital fluorescence microscopy. PLGA scaffolds showed a slight increase in leukocyte recruitment compared to controls. This was associated with an elevation of microvascular permeability, which was comparable to that observed in isogeneic bone tissue. Of interest, PLGA induced a marked angiogenic response, revealing a density of newly formed capillaries almost similar to that observed in bone implants. Histology showed infiltration of macrophages, probably indicating resorption of the biomaterial. In contrast, hydrogel scaffolds induced a severe inflammation, as indicated by an ~15-fold increase of leukocyte–endothelial cell interaction and a marked elevation of microvascular permeability. This was associated by induction of apoptotic cell death within the surrounding tissue and a complete lack of ingrowth of newly formed microvessels. Histology confirmed adequate engraftment of PLGA and isogeneic bone but not hydrogel within the host tissue. PLGA scaffolds show a better biocompatibility than hydrogel scaffolds and promote vascular ingrowth, guaranteeing adequate engraftment within the host tissue.

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Li, Jia Ping, et al. "**Bone ingrowth in porous titanium implants produced by 3D fiber deposition.**" *Biomaterials* 28.18 (2007): 2810-2820.

3D fiber deposition is a technique that allows the development of metallic scaffolds with accurately controlled pore size, porosity and interconnecting pore size, which in turn permits a more precise investigation of the effect of structural properties on the in vivo behavior of biomaterials.
This study analyzed the in vivo performance of titanium alloy scaffolds fabricated using 3D fiber deposition. The titanium alloy scaffolds with different structural properties, such as pore size, porosity and interconnecting pore size were implanted on the decorticated transverse processes of the posterior lumbar spine of 10 goats. Prior to implantation, implant structure and permeability were characterized. To monitor the bone formation over time, fluorochrome markers were administered at 3, 6 and 9 weeks and the animals were sacrificed at 12 weeks after implantation. Bone formation in the scaffolds was investigated by histology and histomorphometry of non-decalcified sections using traditional light- and epifluorescent microscopy. In vivo results showed that increase of porosity and pore size, and thus increase of permeability of titanium alloy implants positively influenced their osteoconductive properties.

Li, JiaPing, et al. "**Biological performance in goats of a porous titanium alloy–biphasic calcium phosphate composite.**" *Biomaterials* 28.29 (2007): 4209-4218.

In this study, porous 3D fiber deposition titanium (3DFT) and 3DFT combined with porous biphasic calcium phosphate ceramic (3DFT+BCP) implants, both bare and 1 week cultured with autologous bone marrow stromal cells (BMSCs), were implanted intramuscularly and orthotopically in 10 goats. To assess the dynamics of bone formation over time, fluorochrome markers were administered at 3, 6 and 9 weeks and the animals were sacrificed at 12 weeks after implantation. New bone in the implants was investigated by histology and histomorphometry of non-decalcified sections. Intramuscularly, no bone formation was found in any of the 3DFT implants, while a very limited amount of bone was observed in 2 BMSC 3DFT implants. 3DFT+BCP and BMSC 3DFT+BCP implants showed ectopic bone formation, in 8 and 10 animals, respectively. The amount of formed bone was significantly higher in BMSC 3DFT+BCP as compared to 3DFT+BCP implants. Implantation on transverse processes resulted in significantly more bone formation in composite structure as compared to titanium alloy alone, both with and without cells. Unlike intramuscularly, the presence of BMSC did not have a significant effect on the amount of new bone either in metallic or in composite structure. Although the 3DFT is inferior to BCP for bone growth, the reinforcement of the brittle BCP with a 3DFT cage did not negatively influence osteogenesis, osteoinduction and osteoconduction as previously shown for the BCP alone. The positive effect of BMSCs was observed ectopically, while it was not significant orthotopically.

Moroni, Lorenzo, et al. "**Anatomical 3D fiber-deposited scaffolds for tissue engineering: designing a neotrachea.**" *Tissue Engineering* 13.10 (2007): 2483-2493.

The advantage of using anatomically shaped scaffolds as compared to modeled designs was investigated and assessed in terms of cartilage formation in an artificial tracheal construct. Scaffolds were rapid prototyped with a technique named three-dimensional fiber deposition (3DF). Anatomical scaffolds were fabricated from a patient-derived computerized tomography dataset, and compared to cylindrical and toroidal tubular scaffolds. Lewis rat tracheal chondrocytes were seeded on 3DF scaffolds and cultured for 21 days. The 3-(4,5-dimethylthiazol-2yl)-2,5-dyphenyltetrazolium bromide (MTT) and sulfated glycosaminoglycan (GAG) assays were performed to measure the relative number of cells and the extracellular matrix (ECM) formed. After 3 weeks of culture, the anatomical scaffolds revealed a significant increase in ECM synthesis and a higher degree of differentiation as shown by the GAG/MTT ratio and by scanning electron microscopy analysis. Interestingly, a lower scaffold's pore volume and porosity resulted in more tissue formation and a better cell differentiation, as evidenced by GAG and GAG/MTT values. Scaffolds were compliant and did not show any signs of luminal obstruction in vitro. These results may promote anatomical scaffolds as functional matrices for tissue regeneration not only to help regain the original shape, but also for their improved capacity to support larger tissue formation.

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Wagner, M., et al. "**Comparative in vitro study of the cell proliferation of ovine and human osteoblast-like cells on conventionally and rapid prototyping produced scaffolds tailored for application as potential bone replacement material.**" *Journal of Biomedical Materials Research Part A* 83.4 (2007): 1154-1164.

Reconstruction of bone defects in the field of craniomaxillofacial surgery is a relevant problem. In regenerative medicine, autologous bone is not available sufficiently. The full replacement of autologous bone grafts is required. A promising research field is the bone engineering. Especially the application of rapid prototyping (RP) enables new perspectives concerning the scaffold design. The aim of the study was to compare scaffolds produced by RP-technology (native and plasma-coated PLGA-scaffolds) with conventionally produced scaffolds (agar plates with hydroxyapatite and hyaluronic acid coated agar plates with hydroxyapatite) relating to proliferation, adhesion, and morphology of osteoblasts to get knowledge about the application potential of such 3D-manufactured matrices for bone engineering. TissueFoil E served as reference. To compare the scaffolds, 12 ovine and 12 human osteoblast-like cell cultures of the skull were used. Results were obtained by EZ4U, scanning electron microscopy, and light microscopy. The highest cell proliferation rate of human osteoblast-like cells was measured on TissueFoil E followed by plasma-coated PLGA-scaffolds and uncoated PLGA-scaffolds, whereas of ovine osteoblast-like cells on plasma-coated PLGA-scaffolds followed by TissueFoil E and uncoated PLGA-scaffolds. Human and ovine osteoblast-like cells on coated and uncoated agar plates had significant lower proliferation rates compared with TissueFoil E and PLGA-scaffolds. These results showed the potential of RP in the field of bone engineering. Mechanical properties of such scaffolds and in vivo studies should be investigated to examine if the scaffolds hold up the pressure it will undergo long enough to allow regrowth of bone and to examine the revascularization.

2008

Al-Ahmad, A., et al. "**Bacterial and Candida albicans adhesion on rapid prototyping-produced 3D-scaffolds manufactured as bone replacement materials.**" *Journal of Biomedical Materials Research Part A* 87.4 (2008): 933-943.

Rapid prototyping (RP)-produced scaffolds aregaining increasing importance in scaffold-guided tissueengineering. Microbial adhesion on the surface of replacement materials has a strong influence on healing and long-term outcome. Consequently, it is important to examine the adherence of microorganisms on RP-produced scaffolds. This research focussed on manufacturing of scaffolds by 3D-bioplotting and examination of their microbial adhesion characteristics. Tricalciumphosphate (TCP), calcium/sodium alginate, and poly(lactide-co-glycolic acid) (PLGA) constructs were produced and used to study the adhesion of dental pathogens. Six oral bacterial strains, one Candida strain and human saliva were used for the adhesion studies. The number of colony forming units (CFU) were determined and scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) were performed. Microorganisms adhered to all scaffolds. All strains, except for Streptococcusoralis, adhered best to PLGA scaffolds. Streptococcusoralis adhered to each of the biomaterials equally. Streptococcusmutans and Enterococcus faecalis adhered best to PLGA scaffolds, followed by alginate and TCP. Prevotella nigrescens, Porphyromonasgingivalis, Streptococcussanguis, and Candida albicans showed the highest adherence to PLGA, followed by TCP and alginate. In contrast, the microorganisms of saliva adhered significantly better to TCP, followed by PLGA and alginate. SEM observations correlated with the results of the CFU determinations. CLSM detected bacteria within deeper sheets of alginate. In conclusion, because of the high adherence rate of oral pathogens to the scaffolds, the application of these biomaterials for bone replacement in oral surgery could result in biomaterial-related infections. Strategies to decrease microbial adherence and to prevent infections due to oral pathogens are discussed.

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El-Ayoubi, Rouwayda, et al. "**Design and fabrication of 3D porous scaffolds to facilitate cell-based gene therapy.**" *Tissue Engineering Part A* 14.6 (2008): 1037-1048.

Biomaterials capable of efficient gene delivery by embedded cells provide a fundamental tool for the treatment of acquired or hereditary diseases. A major obstacle is maintaining adequate nutrient and oxygen diffusion to cells within the biomaterial. In this study, we combined the solid free-form fabrication and porogen leaching techniques to fabricate three-dimensional scaffolds, with bimodal pore size distribution, for cell-based gene delivery. The objective of this study was to design micro-/macroporous scaffolds to improve cell viability and drug delivery. Murine bone marrow-derived mesenchymal stromal cells (MSCs) genetically engineered to secrete erythropoietin (EPO) were seeded onto poly-l-lactide (PLLA) scaffolds with different microporosities. Over a period of 2 weeks in culture, an increase in cell proliferation and metabolic activity was observed with increasing scaffold microporosity. The concentration of EPO detected in supernatants also increased with increasing microporosity level. Our study shows that these constructs can promote cell viability and release of therapeutic proteins, and clearly demonstrates their capacity for a dual role as scaffolds for tissue regeneration and as delivery systems for soluble gene products.

Fedorovich, Natalja E., et al. "**Three-dimensional fiber deposition of cell-laden, viable, patterned constructs for bone tissue printing.**" *Tissue Engineering Part A* 14.1 (2008): 127-133.

Organ or tissue printing, a novel approach in tissue engineering, creates layered, cell-laden hydrogel scaffolds with a defined three-dimensional (3D) structure and organized cell placement. In applying the concept of tissue printing for the development of vascularized bone grafts, the primary focus lies on combining endothelial progenitors and bone marrow stromal cells (BMSCs). Here we characterize the applicability of 3D fiber deposition with a plotting device, Bioplotter, for the fabrication of spatially organized, cell-laden hydrogel constructs. The viability of printed BMSCs was studied in time, in several hydrogels, and extruded from different needle diameters. Our findings indicate that cells survive the extrusion and that their subsequent viability was not different from that of unprinted cells. The applied extrusion conditions did not affect cell survival, and BMSCs could subsequently differentiate along the osteoblast lineage. Furthermore, we were able to combine two distinct cell populations within a single scaffold by exchanging the printing syringe during deposition, indicating that this 3D fiber deposition system is suited for the development of bone grafts containing multiple cell types.

Jukes, Jojanneke M., et al. "**Critical steps toward a tissue-engineered cartilage implant using embryonic stem cells.**" *Tissue Engineering Part A* 14.1 (2008): 135-147.

Embryonic stem (ES) cells are a potential source for cartilage tissue engineering because they provide an unlimited supply of cells that can be differentiated into chondrocytes. So far, chondrogenic differentiation of both mouse and human ES cells has only been demonstrated in two-dimensional cultures, in pellet cultures, in a hydrogel, or on thin biomaterials. The next challenge will be to form cartilage on a load-bearing, clinically relevant–sized scaffold in vitro and in vivo, to regenerate defects in patients suffering from articular cartilage disorders. For a successful implant, cells have to be seeded efficiently and homogenously throughout the scaffold. Parameters investigated were the scaffold architecture, seeding method, and cellular condition. Seeding in a three-dimensional fiber-deposited (3DF) scaffold was more homogenous than in a compression-molded scaffold. The seeding efficiency on bare scaffolds was compromised by the absence of serum in the chondrogenic medium, but could be improved by combining the cells with a gel and subsequent injection into the 3DF scaffolds. However, the viability of the cells was unsatisfactory in the interior of the graft. Cell aggregates, the so-called embryoid bodies (EBs), were seeded with increased survival rate. Mouse ES cells readily underwent chondrogenic differentiation in vitro in pellets, on bare scaffolds, in Matrigel, and in agarose, both as single cells and in EBs. The differentiation protocol requires further improvement to achieve homogenous differentiation and abolish teratoma formation in vivo. We conclude that ES cells can be used as a cell source for cartilage tissue engineering, pending further optimization of the strategy.

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Kyriakidou, K., et al. "**Dynamic co-seeding of osteoblast and endothelial cells on 3D polycaprolactone scaffolds for enhanced bone tissue engineering.**" *Journal of Bioactive and Compatible Polymers* 23.3 (2008): 227-243.

Tissue engineered scaffolds must have an organized and repeatable microstructure which enables cells to assemble in an ordered matrix that allows adequate nutriental perfusion. In this work, to evaluate the reciprocal cell interactions of endothelial and osteoblast-like cells, human osteoblast-like cells (MG63) and Human Umbilical Vein Endothelial Cells (HUVEC) were co-seeded onto 3D geometrically controlled porous poly(ε-caprolactone) (PCL) and cultured by means of a rotary cell culture system (RCCS-4DQ). In our dynamic co-culture system, the lack of significant enhancement of osteoblast ALP activity and ECM production indicated that the microgravity conditions of the rotary system affected the cells by favoring their proliferation and cellular cross-talk. These results emphasize how osteoblasts increase endothelial cell proliferate and endothelial cells amplify the growth of osteoblasts but decrease their differentiation. This dynamic seeding of osteoblasts and endothelial cells onto a 3D polymeric scaffold may represent a unique approach for studying the mechanisms of interaction of endothelial and osteoblast cells as well as achieve a functional hybrid in which angiogenesis, furnished by neo-vascular organization of endothelial cells may further support osteoblasts growth. Furthermore, this in vitro model may be useful in examining the applicability of novel material structures for tissue engineering.

Laschke, Matthias W., et al. "**Improvement of vascularization of PLGA scaffolds by inosculation of in situ-preformed functional blood vessels with the host microvasculature.**" *Annals of surgery* 248.6 (2008): 939-948.

Objective: We analyzed, in vivo, whether the establishment of blood supply to implanted scaffolds can be accelerated by inosculation of an in situ-preformed microvascular network with the host microvasculature.
Background: A rapid vascularization is crucial for the survival of scaffold-based transplanted tissue constructs.
Methods: Poly-lactic-glycolic acid scaffolds were implanted into the flank of balb/c or green fluorescent protein (GFP)-transgenic mice for 20 days to create in situ a new microvascular network within the scaffolds. The prevascularized scaffolds were then transferred into the dorsal skinfold chamber of isogeneic recipient mice. Nonvascularized poly-lactic-glycolic acid scaffolds served as controls. Vascularization, blood perfusion, and cell survival of the implants were analyzed over 14 days using intravital fluorescence microscopy, histology, and immunohistochemistry.
Results: Our results demonstrate that establishment of blood perfusion of prevascularized scaffolds is significantly accelerated and improved (136.7 ± 23.2 pl/s) when compared with controls (6.9 ± 1.9 pl/s), because the in situ-preformed microvessels were reperfused by forming interconnections to the host microvasculature. Apoptotic cell death within the implants was found only during the first 3 to 6 days after scaffold implantation during lack of blood perfusion, but not during the further 14-day observation period.
Conclusions: Inosculation of in situ-preformed functional blood vessels represents a promising approach to improve the blood supply to implanted tissue constructs.

Laschke, M. W., et al. "**Incorporation of growth factor containing Matrigel promotes vascularization of porous PLGA scaffolds.**" *Journal of Biomedical Materials Research Part A* 85.2 (2008): 397-407.

In tissue engineering, rapid ingrowth of blood vessels into scaffolds is a major prerequisite for the survival of three-dimensional tissue constructs. In the present study, we investigated whether the vascularization of implanted poly-D,L-lactic-co-glycolic acid (PLGA) scaffolds may be accelerated by incorporation of Matrigel. For this purpose, we investigated in the aortic ring assay the proangiogenic properties of growth factor reduced Matrigel (GFRM) and growth factor containing Matrigel (GFCM), which were then incorporated into the pores of PLGA scaffolds. Subsequently, we analyzed

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vascularization, biocompatibility, and incorporation of these scaffolds during 14 days after implantation into dorsal skinfold chambers of balb/c mice by means of intravital microscopy, histology, and immunohistochemistry. Matrigel-free scaffolds served as controls. In the aortic ring assay, GFCM stimulated the development of a network of tubular vessel structures with a significantly increased sprout area and density when compared with GFRM. Accordingly, GFCM accelerated and improved in vivo the ingrowth of new blood vessels into scaffolds, resulting in the formation of a pericyte-coated vascular network with an increased functional capillary density in comparison to the GFRM and control group. Besides, analysis of leukocyte–endothelial cell interaction in host tissue venules located in close vicinity to the scaffolds showed no marked differences in numbers of rolling and adherent leukocytes between the observation groups, indicating that incorporation of Matrigel did not affect biocompatibility of PLGA scaffolds. These findings demonstrate that the combination of proangiogenic extracellular matrices with solid scaffold biomaterials may represent a novel approach to accelerate adequate vascularization of tissue engineering constructs.

Moroni, Lorenzo, et al. "**3D Fiber-Deposited Electrospun Integrated Scaffolds Enhance Cartilage Tissue Formation.**" *Advanced Functional Materials* 18.1 (2008): 53-60.

Despite the periodical and completely interconnected pore network that characterizes rapid prototyped scaffolds, cell seeding efficiency remains still a critical factor for optimal tissue regeneration. This can be mainly attributed to the current resolution limits in pore size. We present here novel three-dimensional (3D) scaffolds fabricated by combining 3D fiber deposition (3DF) and electrospinning (ESP). Scaffolds consisted of integrated 3DF periodical macrofiber and random ESP microfiber networks (3DFESP). The 3DF scaffold provides structural integrity and mechanical properties, while the ESP network works as a “sieving” and cell entrapment system and offers?at the same time?cues at the extracellular matrix (ECM) scale. Primary bovine articular chondrocytes were isolated, seeded, and cultured for four weeks on 3DF and 3DFESP scaffolds to evaluate the influence of the integrated ESP network on cell entrapment and on cartilage tissue formation. 3DFESP scaffolds enhanced cell entrapment as compared to 3DF scaffolds. This was accompanied by a higher amount of ECM (expressed in terms of sulphated glycosaminoglycans or GAG) and a significantly higher GAG/DNA ratio after 28 days. SEM analysis revealed rounded cell morphology on 3DFESP scaffolds. Spread morphology was observed on 3DF scaffolds, suggesting a direct influence of fiber dimensions on cell differentiation. Furthermore, the ESP surface topology also influenced cell morphology. Thus, the integration of 3DF and ESP techniques provide a new set of “smart” scaffolds for tissue engineering applications.

Rücker, Martin, et al. "**Vascularization and biocompatibility of scaffolds consisting of different calcium phosphate compounds.**" *Journal of Biomedical Materials Research Part A* 86.4 (2008): 1002-1011.

Scaffolds for tissue engineering of bone should mimic bone matrix and promote vascular ingrowth. Whether synthetic hydroxyapatite and acellular dentin, both materials composed from calcium phosphate, fulfill these material properties has not been studied yet. Therefore, we herein studied in vivo the host angiogenic and inflammatory response to these biomaterials. Porous scaffolds of hydroxyapatite and isogeneic acellular dentin were implanted into the dorsal skinfold chamber of balb/c mice. Additional animals received perforated implants of isogeneic calvarial bone displaying pores similar in size and structure to those of both scaffolds. Chambers of animals without implants served as controls. Angiogenesis and neovascularization as well as inflammatory leukocyte-endothelial cell interaction and microvascular leakage were analyzed over a 14-day time period using intravital fluorescence microscopy. Implantation of both hydroxyapatite and dentin scaffolds showed a slight increase in leukocyte recruitment compared with controls. This was associated with an elevation of microvascular permeability, which was comparable to that observed in response to isogeneic bone. In addition, hydroxyapatite as well as dentin scaffolds induced a marked angiogenic response, which resulted in complete vascularization of the implants until day 14. Of interest, in hydroxyapatite scaffolds, the newly formed capillaries were not as densely meshed as in dentin scaffolds, in which the functional capillary density was comparable to that measured in bone implants. Hydroxyapatite and, in particular,

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dentin scaffolds promote vascularization and exhibit a biocompatibility comparable to that of isogeneic bone. This may guarantee the rapid incorporation of these materials into the host tissue.

Yilgor, Pinar, et al. "**3D plotted PCL scaffolds for stem cell based bone tissue engineering.**" *Macromolecular Symposia*. Vol. 269. No. 1. WILEY-VCH Verlag, 2008.

The ability to control the architecture and strength of a bone tissue engineering scaffold is critical to achieve a harmony between the scaffold and the host tissue. Rapid prototyping (RP) technique is applied to tissue engineering to satisfy this need and to create a scaffold directly from the scanned and digitized image of the defect site. Design and construction of complex structures with different shapes and sizes, at micro and macro scale, with fully interconnected pore structure and appropriate mechanical properties are possible by using RP techniques. In this study, RP was used for the production of poly(ε-caprolactone) (PCL) scaffolds. Scaffolds with four different architectures were produced by using different configurations of the fibers (basic, basic-offset, crossed and crossed-offset) within the architecture of the scaffold. The structure of the prepared scaffolds were examined by scanning electron microscopy (SEM), porosity and its distribution were analyzed by micro-computed tomography (μ-CT), stiffness and modulus values were determined by dynamic mechanical analysis (DMA). It was observed that the scaffolds had very ordered structures with mean porosities about 60%, and having storage modulus values about 1×107 Pa. These structures were then seeded with rat bone marrow origin mesenchymal stem cells (MSCs) in order to investigate the effect of scaffold structure on the cell behavior; the proliferation and differentiation of the cells on the scaffolds were studied. It was observed that cell proliferation was higher on offset scaffolds (262000 vs 235000 for basic, 287000 vs 222000 for crossed structure) and stainings for actin filaments of the cells reveal successful attachment and spreading at the surfaces of the fibers. Alkaline phosphatase (ALP) activity results were higher for the samples with lower cell proliferation, as expected. Highest MSC differentiation was observed for crossed scaffolds indicating the influence of scaffold structure on cellular activities.

2009

El-Ayoubi, Rouwayda, et al. "**Design and Dynamic Culture of 3D Scaffolds for Cartilage Tissue Engineering.**" *Journal of Biomaterials Applications* (2009).

Engineered scaffolds for tissue-engineering should be designed to match the stiffness and strength of healthy tissues while maintaining an interconnected pore network and a reasonable porosity. In this work, we have used 3D-ploting technique to produce poly-LLactide (PLLA) macroporous scaffolds with two different pore sizes. The ability of these macroporous scaffolds to support chondrocyte attachment and viability were compared under static and dynamic loading in vitro. Moreover, the 3D-plotting technique was combined with porogen-leaching, leading to micro/macroporous scaffolds, so as to examine the effect of microporosity on the level of cell attachment and viability under similar loading condition. Canine chondrocytes cells were seeded onto the scaffolds with different topologies, and the constructs were cultured for up to 2 weeks under static conditions or in a bioreactor under dynamic compressive strain of 10% strain, at a frequency of 1 Hz. The attachment and cell growth of chondrocytes were examined by scanning electron microscopy (SEM) and by MTT assay. A significant difference in cell attachment was observed in macroporous scaffolds with different pore sizes after one, 7, and 14 days. Cell viability in the scaffolds was enhanced with decreasing pore size and increasing microporosity level throughout the culture period. Chondrocyte viability in the scaffolds cultured under dynamic loading was significantly higher ($p<0.05$) than the scaffolds cultured statically. Dynamic cell culture of the scaffolds improved cell viability and decrease the time of in vitro culture when compared to statically cultured constructs. Optimizing culture conditions and scaffold properties could generate optimal tissue/constructs combination for cartilage repair.

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Fedorovich, Natalja E., et al. "**Evaluation of photocrosslinked lutrol hydrogel for tissue printing applications.**" *Biomacromolecules* 10.7 (2009): 1689-1696.

Application of hydrogels in tissue engineering and innovative strategies such as organ printing, which is based on layered 3D deposition of cell-laden hydrogels, requires design of novel hydrogel matrices. Hydrogel demands for 3D printing include: 1) preservation of the printed shape after the deposition; 2) maintaining cell viability and cell function and 3) easy handling of the printed construct. In this study we analyze the applicability of a novel, photosensitive hydrogel (Lutrol) for printing of 3D structured bone grafts. We benefit from the fast temperature-responsive gelation ability of thermosensitive Lutrol-F127, ensuring organized 3D extrusion, and the additional stability provided by covalent photocrosslinking allows handling of the printed scaffolds. We studied the cytotoxicity of the hydrogel and osteogenic differentiation of embedded osteogenic progenitor cells. After photopolymerization of the modified Lutrol hydrogel, cells remain viable for up to three weeks and retain the ability to differentiate. Encapsulation of cells does not compromise the mechanical properties of the formed gels and multilayered porous Lutrol structures were successfully printed.

Lee, Chang H., et al. "**Tissue formation and vascularization in anatomically shaped human joint condyle ectopically in vivo.**" *Tissue Engineering Part A* 15.12 (2009): 3923-3930.

Scale-up of bioengineered grafts toward clinical applications is a challenge in regenerative medicine. Here, we report tissue formation and vascularization of anatomically shaped human tibial condyles ectopically with a dimension of $20 \times 15 \times 15\ mm^3$. A composite of poly-ε-caprolactone and hydroxyapatite was fabricated using layer deposition of three-dimensional interlaid strands with interconnecting microchannels (400 μm) and seeded with human bone marrow stem cells (hMSCs) with or without osteogenic differentiation. An overlaying layer (1 mm deep) of poly(ethylene glycol)-based hydrogel encapsulating hMSCs or hMSC-derived chondrocytes was molded into anatomic shape and anchored into microchannels by gel infusion. After 6 weeks of subcutaneous implantation in athymic rats, hMSCs generated not only significantly more blood vessels, but also significantly larger-diameter vessels than hMSC-derived osteoblasts, although hMSC-derived osteoblasts yielded mineralized tissue in microchannels. Chondrocytes in safranin-O-positive glycosaminoglycan matrix were present in the cartilage layer seeded with hMSC-derived chondrogenic cells, although significantly more cells were present in the cartilage layer seeded with hMSCs than hMSC-derived chondrocytes. Together, MSCs elaborate substantially more angiogenesis, whereas their progenies yield corresponding differentiated tissue phenotypes. Scale up is probable by incorporating a combination of stem cells and their progenies in repeating modules of internal microchannels.

Maher, P. S., et al. "**Construction of 3D biological matrices using rapid prototyping technology.**" *Rapid Prototyping Journal* 15.3 (2009): 204-210.

Purpose – Hydrogels with low viscosities tend to be difficult to use in constructing tissue engineering (TE) scaffolds used to replace or restore damaged tissue, due to the length of time it takes for final gelation to take place resulting in the scaffolds collapsing due to their mechanical instability. However, recent advances in rapid prototyping have allowed for a new technology called bioplotting to be developed, which aims to circumvent these inherent problems. This paper aims to present details of the process.
Design/methodology/approach – The paper demonstrates how by using the bioplotting technique complex 3D geometrical scaffolds with accurate feature sizes and good pore definition can be fabriated for use as biological matrices. PEG gels containing the cell-adhesive RGD peptide sequence were patterned using this method to produce layers of directional microchannels which have a functionalised bioactive surface. Seeding these gels with C2C12 myoblasts showed that the cells responded to the topographical features and aligned themselves along the direction of the channels.
Findings – This process allows plotting of various materials into a media bath containing material of similar rheological properties which can be used to both support the structure as it is dispensed and also to initiate cross-linking of the hydrogel. By controlling concentrations, viscosity and the temperature of

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both the plotting material and the plotting media, the speed of the hydrogel gelation can be enhanced whilst it is cross-linking in the media bath. TE scaffolds have been produced using a variety of materials including poly(ethylene glycol) (PEG), gelatin, alginic acid and agarose at various concentrations and viscosities.

Maher, P. S., et al. "**Formed 3D bio-scaffolds via rapid prototyping technology.**" *4th European Conference of the International Federation for Medical and Biological Engineering*. Springer Berlin Heidelberg, 2009

The construction of biomaterial scaffolds for cell seeding is now seen as the most common approach for producing artificial tissue as compared with cell self-assembly and Acellular matrix techniques. This paper describes the use of synthetic and natural polymeric material shaped into 3D biological matrices by using Rapid Prototyping (RP) technology. Recent advances in RP technology have greatly enhanced the range of biomaterials that can now be constructed into scaffolds, also allowing for maximized control of the pore size and architecture. Bioplotting is one such method which allows the dispensing of various biomaterials into a media bath which has similar rheological properties and acts as mechanical support and in most cases a cross-linking agent to produce high quality scaffolds. This method was used to construct scaffolds using agarose and gelatin with tight interconnecting pores which aim to enhance cell growth. Bioplotting was also used to pattern microchanneled layers in one direction with a PEG gel containing cell adhesive RGD peptide sequence, when seeded with C2C12 myoblasts demonstrated that cells responded to their topographical environment and aligned along the direction of the layered microchannels. This result indicates that this technique can be used to produce 3D scaffolds which aid tissue regeneration for physiologically functional tissue.

Martins, Albino, et al. "**Hierarchical starch-based fibrous scaffold for bone tissue engineering applications.**" *Journal of tissue engineering and regenerative medicine* 3.1 (2009): 37-42.

Fibrous structures mimicking the morphology of the natural extracellular matrix are considered promising scaffolds for tissue engineering. This work aims to develop a novel hierarchical starch-based scaffold. Such scaffolds were obtained by a combination of starch–polycaprolactone micro- and polycaprolactone nano-motifs, respectively produced by rapid prototyping (RP) and electrospinning techniques. Scanning electron microscopy (SEM) and micro-computed tomography analysis showed the successful fabrication of a multilayer scaffold composed of parallel aligned microfibres in a grid-like arrangement, intercalated by a mesh-like structure with randomly distributed nanofibres (NFM). Human osteoblast-like cells were dynamically seeded on the scaffolds, using spinner flasks, and cultured for 7 days under static conditions. SEM analysis showed predominant cell attachment and spreading on the nanofibre meshes, which enhanced cell retention at the bulk of the composed/hierarchical scaffolds. A significant increment in cell proliferation and osteoblastic activity, assessed by alkaline phosphatase quantification, was observed on the hierarchical fibrous scaffolds. These results support our hypothesis that the integration of nanoscale fibres into 3D rapid prototype scaffolds substantially improves their biological performance in bone tissue-engineering strategies.

Oliveira, A. L., et al. "**Nucleation and growth of biomimetic apatite layers on 3D plotted biodegradable polymeric scaffolds: effect of static and dynamic coating conditions.**" *Acta biomaterialia* 5.5 (2009): 1626-1638.

Apatite layers were grown on the surface of newly developed starch/polycaprolactone (SPCL)-based scaffolds by a 3D plotting technology. To produce the biomimetic coatings, a sodium silicate gel was used as nucleating agent, followed by immersion in a simulated body fluid (SBF) solution. After growing a stable apatite layer for 7 days, the scaffolds were placed in SBF under static, agitated (80 strokes min^{-1}) and circulating flow perfusion (Q = 4 ml min^{-1}; $_{tR}$ = 15 s) for up to 14 days. The materials were characterized by scanning electron microscopy/energy dispersive X-ray spectroscopy, Fourier transform

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infrared spectroscopy and thin-film X-ray diffraction. Cross-sections were obtained and the coating thickness was measured. The elemental composition of solution and coatings was monitored by inductively coupled plasma spectroscopy. After only 6 h of immersion in SBF it was possible to observe the formation of small nuclei of an amorphous calcium phosphate (ACP) layer. After subsequent SBF immersion from 7 to 14 days under static, agitated and circulating flow perfusion conditions, these layers grew into bone-like nanocrystalline carbonated apatites covering each scaffold fiber without compromising its initial morphology. No differences in the apatite composition/chemical structure were detectable between the coating conditions. In case of flow perfusion, the coating thickness was significantly higher. This condition, besides mimicking better the biological milieu, allowed for the coating of complex architectures at higher rates, which can greatly reduce the coating step.

Park, SuA, et al. "**3D polycaprolactone scaffolds with controlled pore structure using a rapid prototyping system.**" *Journal of Materials Science: Materials in Medicine* 20.1 (2009): 229-234.

Designing a three-dimensional (3-D) ideal scaffold has been one of the main goals in biomaterials and tissue engineering, and various mechanical techniques have been applied to fabricate biomedical scaffolds used for soft and hard tissue regeneration. Scaffolds should be biodegradable and biocompatible, provide temporary support for cell growth to allow cell adhesion, and consist of a defined structure that can be formed into customized shapes by a computer-aided design system. This versatility in preparing scaffolds gives us the opportunity to use rapid prototyping devices to fabricate polymeric scaffolds. In this study, we fabricated polycaprolactone scaffolds with interconnecting pores using a 3-D melt plotting system and compared the plotted scaffolds to those made by salt leaching. Scanning electron microscopy, a laser scanning microscope, micro-computed tomography, and dynamic mechanical analysis were used to characterize the geometry and mechanical properties of the resulting scaffolds and morphology of attached cells. The plotted scaffolds had the obvious advantage that their mechanical properties could be easily manipulated by adjusting the scaffold geometry. In addition, the plotted scaffolds provided more opportunity for cells to expand between the strands of the scaffold compared to the salt-leached scaffold.

Schumann, Paul, et al. "**Consequences of seeded cell type on vascularization of tissue engineering constructs in vivo.**" *Microvascular research 78.2* (2009): 180-190.

Implantation of tissue engineering constructs is a promising technique to reconstruct injured tissue. However, after implantation the nutrition of the constructs is predominantly restricted to vascularization. Since cells possess distinct angiogenic potency, we herein assessed whether scaffold vitalization with different cell types improves scaffold vascularization. 32 male balb/c mice received a dorsal skinfold chamber. Angiogenesis, microhemodynamics, leukocyte–endothelial cell interaction and microvascular permeability induced in the host tissue after implantation of either collagen coated poly (l-lactide-co-glycolide) (PLGA) scaffolds (group 4), additionally seeded with osteoblast-like cells (OLCs, group 1), bone marrow mesenchymal stem cells (bmMSCs, group 2) or a combination of OLCs and bmMSCs (group 3) were analyzed repetitively over 14 days using intravital fluorescence microscopy. Apart from a weak inflammatory response in all groups, vascularization was found distinctly accelerated in vitalized scaffolds, indicated by a significantly increased microvascular density (day 6, group 1: 202 ± 15 cm/cm2, group 2: 202 ± 12 cm/cm2, group 3: 194 ± 8 cm/cm2), when compared with controls (group 4: 72 ± 5 cm/cm2). This acceleration was independent from the seeded cell type. Immunohistochemistry revealed in vivo VEGF expression in close vicinity to the seeded OLCs and bmMSCs. Therefore, the observed lack of cell type confined differences in the vascularization process suggests that the accelerated vascularization of vitalized scaffolds is VEGF-related rather than dependent on the potential of bmMSCs to differentiate into specific vascular cells.

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Silva, Nuno A., et al. "**Development and characterization of a Novel Hybrid Tissue Engineering–based scaffold for spinal cord injury repair.**" *Tissue Engineering Part A* 16.1 (2009): 45-54.

Spinal cord injury (SCI) represents a significant health and social problem, and therefore it is vital to develop novel strategies that can specifically target it. In this context, the objective of the present work was to develop a new range of three-dimensional (3D) tubular structures aimed at inducing the regeneration within SCI sites. Up to six different 3D tubular structures were initially developed by rapid prototyping: 3D bioplotting–based on a biodegradable blend of starch. These structures were then further complemented by injecting Gellan Gum, a polysaccharide-based hydrogel, in the central area of structures. The mechanical properties of these structures were assessed using dynamic mechanical analysis, under both dry and wet conditions, and their morphologies/porosities were analyzed using micro-computed tomography and scanning electron microscopy. Biological evaluation was carried out to determine their cytotoxicity, using both minimum essential medium (MEM) extraction and MTS tests, as well as by encapsulation of an oligodendrocyte-like cell (M03-13 cell line) within the hydrogel phase. The histomorphometric analysis showed a fully interconnected network of pores with porosity ranging from 70% to 85%. Scaffolds presented compressive modulus ranging from 17.4 to 62.0 MPa and 4.42 to 27.4 MPa under dry and wet conditions, respectively. Cytotoxicity assays revealed that the hybrid starch/poly-ε-caprolactone/Gellan Gum scaffolds were noncytotoxic, as they did not cause major alterations on cell morphology, proliferation, and metabolic viability. Moreover, preliminary cell encapsulation assays showed that the hybrid scaffolds could support the in vitro culture of oligodendrocyte-like cells. Finally, preliminary in vivo studies conducted in a hemisection rat SCI model revealed that the above-referred structures were well integrated within the injury and did not trigger chronic inflammatory processes. The results herein presented indicate that these 3D systems might be of use in future SCI regeneration approaches.

Son, JoonGon, and GeunHyung Kim. "**Three-dimensional plotter technology for fabricating polymeric scaffolds with micro-grooved surfaces.**" *Journal of Biomaterials Science, Polymer Edition* 20.14 (2009): 2089-2101.

Various mechanical techniques have been used to fabricate biomedical scaffolds, including rapid prototyping (RP) devices that operate from CAD files of the target feature information. The three-dimensional (3-D) bio-plotter is one RP system that can produce design-based scaffolds with good mechanical properties for mimicking cartilage and bones. However, the scaffolds fabricated by RP have very smooth surfaces, which tend to discourage initial cell attachment. Initial cell attachment, migration, differentiation and proliferation are strongly dependent on the chemical and physical characteristics of the scaffold surface. In this study, we propose a new 3-D plotting method supplemented with a piezoelectric system for fabricating surface-modified scaffolds. The effects of the physically-modified surface on the mechanical and hydrophilic properties were investigated, and the results of cell culturing of chondrocytes indicate that this technique is a feasible new method for fabricating high-quality 3-D polymeric scaffolds.

Tan, Qiang, et al. "**Intra-scaffold continuous medium flow combines chondrocyte seeding and culture systems for tissue engineered trachea construction.**" *Interactive Cardiovascular and Thoracic Surgery* 8.1 (2009): 27-30.

In this study we tested the possibility of seeding chondrocytes into poly (ethylene glycol)-terephthalate–poly (butylene terephthalate) PEOT/PBT scaffold through an intra-scaffold medium flow and the impact of this continuous medium flow on subsequent chondrocyte-scaffold culture. Eight cubic PEOT/PBT co-polymers (1 cm3) were assigned into two groups. In the semi-dynamic seeding group a continuous medium flow was created inside the scaffolds by a pump system. Around six million chondrocytes were harvested each day, suspended in 1 ml medium and delivered onto the scaffold through the perfusion for a sequential five days. Traditional chondrocytes directly seeding and static culture method was

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performed as control. Scanning electron microscopy (SEM) and histology assessments were performed to evaluate the distribution of chondrocytes inside the scaffolds and MTT test was chosen to check cell vitality. SEM pictures and histology slices from the perfusion group showed a better three-dimensional cell growth and extensive cell distribution inside the scaffolds; while in the control group chondrocytes only dispersedly formed a monolayer on the surface of scaffolds. Accordingly, MTT results from the perfusion group were much higher than those from control group (0.123 vs. 0.067, P<0.01). Continuous medium perfusion inside PEOT/PBT scaffold effectively combines chondrocyte seeding and culture systems for the reconstruction of tissue engineered trachea.

Woodfield, T., et al. "**Cartilage Tissue Engineering Using Smart Scaffold Design & Advanced Bio Manufacturing.**" *Journal of Bone & Joint Surgery, British Volume* 91.SUPP II (2009): 343-343.

Articular cartilage has a limited regenerative capacity. Tissue engineering strategies adopting seeding and differentiation of individual chondrocytes on porous 3D scaffolds of clinically relevant size remains a considerable challenge. A well documented method to produce small samples of differentiated cartilage tissue in vitro is via micro-mass (pellet) culture, whereby, high concentrations of chondrocytes coalesce to form. a spherical tissue pellet. However, pellet culture techniques are not applied clinically as it is only possible to produce small amounts of tissue (1–2mm). The aims of this study were to develop a method for mass-production of pellets, and investigate whether an alternative “pellet seeding” approach using smart 3D scaffold design would allow large numbers of spherical pellets to be fixed in place.

Woodfield, T. B. F., et al. "**Rapid prototyping of anatomically shaped, tissue-engineered implants for restoring congruent articulating surfaces in small joints.**" *Cell proliferation* 42.4 (2009): 485-497.

Background: Preliminary studies investigated advanced scaffold design and tissue engineering approaches towards restoring congruent articulating surfaces in small joints.
Materials and methods: Anatomical femoral and tibial cartilage constructs, fabricated by three-dimensional fibre deposition (3DF) or compression moulding/particulate leaching (CM), were evaluated in vitro and in vivo in an autologous rabbit model. Effects of scaffold pore architecture on rabbit chondrocyte differentiation and mechanical properties were evaluated following in vitro culture and subcutaneous implantation in nude mice. After femoral and tibial osteotomy and autologous implantation of tissue-engineered constructs in rabbit knee joints, implant fixation and joint articulation were evaluated.
Results: Rapid prototyping of 3DF architectures with 100% interconnecting pores promoted homogeneous distribution of viable cells, glycosaminoglycan (GAG) and collagen type II; significantly greater GAG content and differentiation capacity (GAG/DNA) in vitro compared to CM architectures; and higher mechanical equilibrium modulus and dynamic stiffness (at 0.1 Hz). Six weeks after implantation, femoral and tibial constructs had integrated with rabbit bone and knee flexion/extension and partial load bearing were regained. Histology demonstrated articulating surfaces between femoral and tibial constructs for CM and 3DF architectures; however, repair tissue appeared fibrocartilage-like and did not resemble implanted cartilage.
Conclusions: Anatomically shaped, tissue-engineered constructs with designed mechanical properties and internal pore architectures may offer alternatives for reconstruction or restoration of congruent articulating surfaces in small joints.

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Bat, Erhan, et al. "**Ultraviolet light crosslinking of poly (trimethylene carbonate) for elastomeric tissue engineering scaffolds.**" *Biomaterials* 31.33 (2010): 8696-8705.

A practical method of photocrosslinking high molecular weight poly(trimethylene carbonate)(PTMC) is presented. Flexible, elastomeric and biodegradable networks could be readily prepared by UV irradiating PTMC films containing pentaerythritol triacrylate (PETA) and a photoinitiator. The network characteristics, mechanical properties, wettability, and in vitro enzymatic erosion of the photocrosslinked PTMC films were investigated. Densely crosslinked networks with gel contents up to 98% could be obtained in this manner. Upon photocrosslinking, flexible and tough networks with excellent elastomeric properties were obtained. To illustrate the ease with which the properties of the networks can be tailored, blends of PTMC with mPEG-PTMC or with PTMC-PCL-PTMC were also photocrosslinked. The wettability and the enzymatic erosion rate of the networks could be tuned by blending with block copolymers. Tissue engineering scaffolds were also fabricated using these flexible photocrosslinkable materials. After crosslinking, the fabricated PTMC-based scaffolds showed inter-connected pores and extensive microporosity. Human mesenchymal stem cell (hMSC) culturing studies showed that the photocrosslinked scaffolds prepared from PTMC and PTMC/PTMC-PCL-PTMC blends are well-suited for tissue engineering applications.

De Santis, R., et al. "**An approach in developing 3D fiber-deposited magnetic scaffolds for tissue engineering.**" *V INTERNATIONAL CONFERENCE ON TIMES OF POLYMERS (TOP) AND COMPOSITES*. Vol. 1255. No. 1. AIP Publishing, 2010.

Scaffolds should possess suitable properties to play their specific role. In this work, the potential of 3D fiber deposition technique to develop multifunctional and well-defined magnetic poly(ε-caprolactone)/iron oxide scaffolds has been highlighted, and the effect of iron oxide nanoparticles on the biological and mechanical performances has been assessed.

Haberstroh, Kathrin, et al. "**Bone repair by cell-seeded 3D-bioplotted composite scaffolds made of collagen treated tricalciumphosphate or tricalciumphosphate-chitosan-collagen hydrogel or PLGA in ovine critical-sized calvarial defects.**" *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 93.2 (2010): 520-530.

The aim of this study was to investigate the osteogenic effect of three different cell-seeded 3D-bioplotted scaffolds in a ovine calvarial critical-size defect model. The choice of scaffold-materials was based on their applicability for 3D-bioplotting and respective possibility to produce tailor-made scaffolds for the use in cranio-facial surgery for the replacement of complex shaped boneparts. Scaffold raw-materials are known to be osteoinductive when being cell-seeded [poly(L-lactide-co-glycolide) (PLGA)] or having components with osteoinductive properties as tricalciumphosphate (TCP) or collagen (Col) or chitosan. The scaffold-materials PLGA, TCP/Col, and HYDR (TCP/Col/chitosan) were cell-seeded with osteoblast-like cells whether gained from bone (OLB) or from periost (OLP). In a prospective and randomized design nine sheep underwent osteotomy to create four critical-sized calvarial defects. Three animals each were assigned to the HYDR-, the TCP/Col-, or the PLGA-group. In each animal, one defect was treated with a cell-free, an OLB- or OLP-seeded group-specific scaffold, respectively. The fourth defect remained untreated as control (UD). Fourteen weeks later, animals were euthanized for histo-morphometrical analysis of the defect healing. OLB- and OLP-seeded HYDR and OLB-seeded TCP/Col scaffolds significantly increased the amount of newly formed bone (NFB) at the defect bottom and OLP-seeded HYDR also within the scaffold area, whereas PLGA-scaffolds showed lower rates. The relative density of NFB was markedly higher in the HYDR/OLB group compared to the corresponding PLGA group. TCP/Col had good stiffness to prepare complex structures by bioplotting but HYDR and PLGA were very soft. HYDR showed appropriate biodegradation, TCP/Col and PLGA seemed to be nearly undegraded after 14 weeks. 3D-bioplotted, cell-seeded HYDR and TCP/Col scaffolds increased the amount of NFB within ovine critical-size calvarial defects, but stiffness, respectively, biodegradation of

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materials is not appropriate for the application in cranio-facial surgery and have to be improved further by modifications of the manufacturing process or their material composition.

Kim, K., et al. "**Anatomically shaped tooth and periodontal regeneration by cell homing.**" *Journal of Dental Research* 89.8 (2010): 842-847.

Tooth regeneration by cell delivery encounters translational hurdles. We hypothesized that anatomically correct teeth can regenerate in scaffolds without cell transplantation. Novel, anatomically shaped human molar scaffolds and rat incisor scaffolds were fabricated by 3D bioprinting from a hybrid of poly-ε-caprolactone and hydroxyapatite with 200-μm-diameter interconnecting microchannels. In each of 22 rats, an incisor scaffold was implanted orthotopically following mandibular incisor extraction, whereas a human molar scaffold was implanted ectopically into the dorsum. Stromal-derived factor-1 (SDF1) and bone morphogenetic protein-7 (BMP7) were delivered in scaffold microchannels. After 9 weeks, a putative periodontal ligament and new bone regenerated at the interface of rat incisor scaffold with native alveolar bone. SDF1 and BMP7 delivery not only recruited significantly more endogenous cells, but also elaborated greater angiogenesis than growth-factor-free control scaffolds. Regeneration of tooth-like structures and periodontal integration by cell homing provide an alternative to cell delivery, and may accelerate clinical applications.

Lee, Chang H., et al. "**Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study.**" *The Lancet* 376.9739 (2010): 440-448.

Background: A common approach for tissue regeneration is cell delivery, for example by direct transplantation of stem or progenitor cells. An alternative, by recruitment of endogenous cells, needs experimental evidence. We tested the hypothesis that the articular surface of the synovial joint can regenerate with a biological cue spatially embedded in an anatomically correct bioscaffold.
Methods: In this proof of concept study, the surface morphology of a rabbit proximal humeral joint was captured with laser scanning and reconstructed by computer-aided design. We fabricated an anatomically correct bioscaffold using a composite of poly-ε-caprolactone and hydroxyapatite. The entire articular surface of unilateral proximal humeral condyles of skeletally mature rabbits was surgically excised and replaced with bioscaffolds spatially infused with transforming growth factor β3 (TGFβ3)-adsorbed or TGFβ3-free collagen hydrogel. Locomotion and weightbearing were assessed 1–2, 3–4, and 5–8 weeks after surgery. At 4 months, regenerated cartilage samples were retrieved from in vivo and assessed for surface fissure, thickness, density, chondrocyte numbers, collagen type II and aggrecan, and mechanical properties.
Findings: Ten rabbits received TGFβ3-infused bioscaffolds, ten received TGFβ3-free bioscaffolds, and three rabbits underwent humeral-head excision without bioscaffold replacement. All animals in the TGFβ3-delivery group fully resumed weightbearing and locomotion 3–4 weeks after surgery, more consistently than those in the TGFβ3-free group. Defect-only rabbits limped at all times. 4 months after surgery, TGFβ3-infused bioscaffolds were fully covered with hyaline cartilage in the articular surface. TGFβ3-free bioscaffolds had only isolated cartilage formation, and no cartilage formation occurred in defect-only rabbits. TGFβ3 delivery yielded uniformly distributed chondrocytes in a matrix with collagen type II and aggrecan and had significantly greater thickness ($p=0·044$) and density ($p<0·0001$) than did cartilage formed without TGFβ3. Compressive and shear properties of TGFβ3-mediated articular cartilage did not differ from those of native articular cartilage, and were significantly greater than those of cartilage formed without TGFβ3. Regenerated cartilage was avascular and integrated with regenerated subchondral bone that had well defined blood vessels. TGFβ3 delivery recruited roughly 130% more cells in the regenerated articular cartilage than did spontaneous cell migration without TGFβ3.
Interpretation: Our findings suggest that the entire articular surface of the synovial joint can regenerate without cell transplantation. Regeneration of complex tissues is probable by homing of endogenous cells, as exemplified by stratified avascular cartilage and vascularised bone. Whether cell homing acts as an adjunctive or alternative approach of cell delivery for regeneration of tissues with different organisational complexity warrants further investigation.

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Lee, Jun-Hee, et al. "**Fabrication and characterization of 3D scaffold using 3D plotting system.**" *Chinese Science Bulletin* 55.1 (2010): 94-98.

In this paper, we design and fabricate a 3D scaffold using rapid prototyping (RP) technology for tissue engineering. The scaffold should have a three-dimensional interconnected pore network. We fabricate a polycaprolactone (PCL) scaffold with interconnecting pores and uniform porosity for cell ingrowth using a 3D plotting system. In order to keep the three dimensional shape under mechanical loading while implanted, we design an oscillating nozzle system to increase elastic modulus and yield strength of PCL strand. We characterize the influence of pore geometry, compressive modulus of the scaffold, elastic modulus and yield strength of the strand using SEM, dynamical mechanical analysis (DMA) and Nano-UTM. Finally the cell responses on scaffolds are observed.

Li, J. P., et al. "**The effect of scaffold architecture on properties of direct 3D fiber deposition of porous Ti6Al4V for orthopedic implants.**" *Journal of Biomedical Materials Research Part A* 92.1 (2010): 33-42.

3D porous Ti6Al4V scaffolds were directly fabricated by a rapid prototyping technology, 3D fiber deposition (3DF). In this study, scaffolds with different structures were fabricated by changing fiber spacing and fiber orientation. The influence of different architectures on mechanical properties and permeability of the scaffold were investigated. Mechanical analysis revealed that compressive strength and E-modulus increase with decreasing the porosity. Permeability measurements showed that not only the total porosity but also the porous structure can influence the permeability. 3DF was found to provide good control and reproducibility of the desired degree of porosity and the 3D structure. Results of this study demonstrate that the 3DF of Ti6Al4V give us flexibility and versatility to fabricate and improve scaffolds to better mimic the architecture and properties of natural bone and meet the requirements of bone graft substitutes and orthopedic and dental implants.

Lindhorst, Daniel, et al. "**Effects of VEGF loading on scaffold-confined vascularization.**" *Journal of Biomedical Materials Research Part A* 95.3 (2010): 783-792.

Adequate vascularization of tissue-engineered constructs remains a major challenge in bone grafting. In view of this, we loaded ß-tricalcium-phosphate (ß-TCP) and porous poly(L-lactide-co-glycolide) (PLGA) scaffolds via collagen coating with vascular endothelial growth factor (VEGF) and studied whether the VEGF loading improves scaffold angiogenesis and vascularization. Dorsal skinfold chambers were implanted into 48 balb/c mice, which were assigned to 6 groups (n = 8 each). Uncoated (controls), collagen-coated, and additionally VEGF-loaded PLGA and ß-TCP scaffolds were inserted into the chambers. Angiogenesis, neovascularization, and leukocyte-endothelial cell interaction were analyzed repeatedly during a 14-day observation period using intravital fluorescence microscopy. Furthermore, VEGF release from PLGA und ß-TCP scaffolds was studied by ELISA. Micromorphology was studied from histological specimens. Unloaded ß-TCP scaffolds showed an accelerated and increased angiogenic response when compared with unloaded PLGA scaffolds. In vitro, PLGA released significantly higher amounts of VEGF compared with ß-TCP at the first two days resulting in a rapid drop of the released amount at the following days up to day 7 where the VEGF release was negligible. Nonetheless, in vivo VEGF loading increased neovascularization, especially in ß-TCP scaffolds. This increased vascularization was associated with a temporary leukocytic response with pronounced leukocyte-endothelial cell interaction at days 3 and 6. Histology revealed adequate host tissue response and engraftment of both ß-TCP and PLGA scaffolds. Our study demonstrates that ß-TCP scaffolds offer more suitable conditions for vascularization than PLGA scaffolds, in particular if they are loaded with VEGF.

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Tavassol, Frank, et al. "**Accelerated angiogenic host tissue response to poly (L-lactide-co-glycolide) scaffolds by vitalization with osteoblast-like cells.**" *Tissue Engineering Part A* 16.7 (2010): 2265-2279.

Background: Bone substitutes should ideally promote rapid vascularization, which could be accelerated if these substitutes were vitalized by autologous cells. Although adequate engraftment of porous poly(L-lactide-co-glycolide) (PLGA) scaffolds has been demonstrated in the past, it has not yet been investigated how vascularization is influenced by vitalization or, more precisely, by seeding PLGA scaffolds with osteoblast-like cells (OLCs). For this reason, we conducted an in vivo study to assess host angiogenic and inflammatory responses after the implantation of PLGA scaffolds vitalized with isogeneic OLCs.
Materials and Methods: OLCs were seeded on collagen-coated PLGA scaffolds that were implanted into dorsal skinfold chambers in BALB/c mice (n = 8). Two further groups of animals received either collagen-coated (n = 8) or uncoated PLGA scaffolds (n = 8). Animals that received chambers without implants served as controls (n = 8). Angiogenesis, neovascularization, and leukocyte–endothelial cell interaction were analyzed for 14 days using intravital fluorescence microscopy.
Results: PLGA scaffolds with and without OLCs showed a temporary increase in leukocyte recruitment. At day 3 after implantation, a marked angiogenic host tissue response was observed in close vicinity of all scaffolds studied. At days 6 and 10, the angiogenic response was significantly higher ($p < 0.05$) in PLGA scaffolds vitalized with OLCs than in uncoated or collagen-coated PLGA scaffolds. The majority of OLCs, however, died within 14 days after implantation.
Conclusion: Our study demonstrates that PLGA scaffold vitalization with OLCs accelerates the angiogenic response in the surrounding host tissue. Bone substitutes created by tissue engineering may thus be superior to nonvitalized substitutes although the seeded cells do not survive for long periods.

Yilgor, Pinar, et al. "**Effect of scaffold architecture and BMP-2/BMP-7 delivery on in vitro bone regeneration.**" *Journal of Materials Science: Materials in Medicine* 21.11 (2010): 2999-3008.

The aim of this study was to develop 3-D tissue engineered constructs that mimic the in vivo conditions through a self-contained growth factor delivery system. A set of nanoparticles providing the release of BMP-2 initially followed by the release of BMP-7 were incorporated in poly(ε-caprolactone) scaffolds with different 3-D architectures produced by 3-D plotting and wet spinning. The release patterns were: each growth factor alone, simultaneous, and sequential. The orientation of the fibers did not have a significant effect on the kinetics of release of the model protein BSA; but affected proliferation of bone marrow mesenchymal stem cells. Cell proliferation on random scaffolds was significantly higher compared to the oriented ones. Delivery of BMP-2 alone suppressed MSC proliferation and increased the ALP activity to a higher level than that with BMP-7 delivery. Proliferation rate was suppressed the most by the sequential delivery of the two growth factors from the random scaffold on which the ALP activity was the highest. Results indicated the distinct effect of scaffold architecture and the mode of growth factor delivery on the proliferation and osteogenic differentiation of MSCs, enabling us to design multifunctional scaffolds capable of controlling bone healing.

Yoon, Hyeon, Geun Hyung Kim, and Young Ho Koh. "**A micro-scale surface-structured PCL scaffold fabricated by a 3D plotter and a chemical blowing agent.**" *Journal of Biomaterials Science, Polymer Edition* 21.2 (2010): 159-170.

To study cell responses, polymeric scaffolds with a controllable pore size and porosity have been fabricated using rapid-prototyping methods. However, the scaffolds fabricated by rapid prototyping have very smooth surfaces, which tend to discourage initial cell attachment. Initial cell attachment, migration, differentiation and proliferation are strongly dependent on the chemical and physical characteristics of the scaffold surface. In this study, we propose a three-dimensional (3D) plotting

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method supplemented with a chemical blowing agent to produce a surface-modified 3D scaffold in which the surface is inscribed with nano- and micro-sized pores. The chemically-blown 3D polymeric scaffold exhibited positive qualities, including the compressive modulus, hydrophilicity and initial cell adhesion. Cell cultures on the scaffolds demonstrated that chondrocytes interacted better with the surface-modified scaffold than with a normal 3D scaffold.

Al-Ahmad, A., et al. "**Comparison of bacterial adhesion and cellular proliferation on newly developed three-dimensional scaffolds manufactured by rapid prototyping technology.**" *Journal of Biomedical Materials Research Part A* 98.2 (2011): 303-311.

Scaffolds used in the field of tissue engineering should facilitate the adherence, spreading, and ingrowth of cells as well as prevent microbial adherence. For the first time, this study simultaneously deals with microbial and tissue cell adhesion to rapid prototyping-produced 3D-scaffolds. The cell growth of human osteosarcoma cells (CAL-72) over a time period of 3–11 days were examined on three scaffolds (PLGA, PLLA, PLLA-TCP) and compared to the adhesion of salivary microorganisms and representative germs of the oral flora (Porphyromonas gingivalis, Prevotella nigrescens, Candida albicans, Enterococcus faecalis, Streptococcus mutans, and Streptococcus sanguinis). Scanning electron microscopy (SEM), cell proliferation measurements, and determination of the colony forming units (CFU) were performed. The cell proliferation rates on PLLA and PLLA-TCP after 3, 7, and 11 days of cultivation were higher than on PLGA. On day 3 the proliferation rates on PLLA and PLLA-TCP, and on day 5 on PLLA-TCP, proved to be significantly higher compared to that of the control (culture plate). The strain which showed the most CFUs on all of the investigated scaffolds was P. gingivalis, followed by E. faecalis. No significant CFU differences were determined examining P. gingivalis among the biomaterials. In contrast, E. faecalis was significantly more adherent to PLGA and PLLA compared to PLLA-TCP. The lowest CFU values were seen with C. albicans and P. nigrescens. Salivary born aerobic and anaerobic microorganisms adhered significantly more to PLGA compared to PLLA-TCP. These results supported by SEM point out the high potential of PLLA-TCP in the field of tissue engineering.

Banobre-Lopez, M., et al. "**Poly (caprolactone) based magnetic scaffolds for bone tissue engineering.**" *Journal of Applied Physics* 109.7 (2011): 07B313.

Synthetic scaffolds for tissue engineering coupled to stem cells represent a promising approach aiming to promote the regeneration of large defects of damaged tissues or organs. Magnetic nanocomposites formed by a biodegradable poly(caprolactone) (PCL) matrix and superparamagneticiron doped hydroxyapatite (FeHA) nanoparticles at different PCL/FeHA compositions have been successfully prototyped, layer on layer, through 3D bioplotting. Magnetic measurements, mechanical testing, and imaging were carried out to calibrate both model and technological processing in the magnetized scaffold prototyping. An amount of 10% w/w of magnetic FeHA nanoparticles represents a reinforcement for PCL matrix, however, a reduction of strain at failure is also observed. Energy loss (absorption) measurements under a radio-frequency applied magnetic field were performed in the resulting magnetic scaffolds and very promising heating properties were observed, making them very useful for potential biomedical applications.

Bettahalli, N. M. S., et al. "**Integration of hollow fiber membranes improves nutrient supply in three-dimensional tissue constructs.**" *Acta Biomaterialia* 7.9 (2011): 3312-3324.

Sufficient nutrient and oxygen transport is a potent modulator of cell proliferation in in vitro tissue-engineered constructs. The lack of oxygen and culture medium can create a potentially lethal environment and limit cellular metabolic activity and growth. Diffusion through scaffold and multi-cellular tissue typically limits transport in vitro, leading to potential hypoxic regions and reduction in the viable tissue thickness. For the in vitro generation of clinically relevant tissue-engineered grafts, current nutrient diffusion limitations should be addressed. Major approaches to overcoming these include culture with bioreactors, scaffolds with artificial microvasculature, oxygen carriers and pre-

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vascularization of the engineered tissues. This study focuses on the development and utilization of a new perfusion culture system to provide adequate nutrient delivery to cells within large three-dimensional (3D) scaffolds. Perfusion of oxygenated culture medium through porous hollow fiber (HF) integrated within 3D free form fabricated (FFF) scaffolds is proposed. Mouse pre-myoblast (C2C12) cells cultured on scaffolds of poly(ethylene-oxide-terephthalate)–poly(butylene-terephthalate) block copolymer (300PEOT55PBT45) integrated with porous HF membranes of modified poly(ether-sulfone) (mPES, Gambro GmbH) is used as a model system. Various parameters such as fiber transport properties, fiber spacing within a scaffold and medium flow conditions are optimized. The results show that four HF membranes integrated with the scaffold significantly improve the cell density and cell distribution. This study provides a basis for the development of a new HF perfusion culture methodology to overcome the limitations of nutrient diffusion in the culture of large 3D tissue constructs.

Coutu, Daniel L., et al. "**Hierarchical scaffold design for mesenchymal stem cell-based gene therapy of hemophilia B.**" *Biomaterials* 32.1 (2011): 295-305.

Gene therapy for hemophilia B and other hereditary plasma protein deficiencies showed great promise in pre-clinical and early clinical trials. However, safety concerns about in vivo delivery of viral vectors and poor post-transplant survival of ex vivo modified cells remain key hurdles for clinical translation of gene therapy. We here describe a 3D scaffold system based on porous hydroxyapatite–PLGA composites coated with biomineralized collagen 1. When combined with autologous gene-engineered factor IX (hFIX) positive mesenchymal stem cells (MSCs) and implanted in hemophilic mice, these scaffolds supported long-term engraftment and systemic protein delivery by MSCs in vivo. Optimization of the scaffolds at the macro-, micro- and nanoscales provided efficient cell delivery capacity, MSC self-renewal and osteogenesis respectively, concurrent with sustained delivery of hFIX. In conclusion, the use of gene-enhanced MSC-seeded scaffolds may be of practical use for treatment of hemophilia B and other plasma protein deficiencies.

De Santis, R., et al. "**A basic approach toward the development of nanocomposite magnetic scaffolds for advanced bone tissue engineering.**" *Journal of Applied Polymer Science* 122.6 (2011): 3599-3605.

Magnetic scaffolds for bone tissue engineering based on a poly(ε-caprolactone) (PCL) matrix and iron oxide (Fe_3O_4) magnetic nanoparticles were designed and developed through a three-dimensional (3D) fiber-deposition technique. PCL/Fe_3O_4 scaffolds were characterized by a 90/10 w/w composition. Tensile and magnetic measurements were carried out, and nondestructive 3D imaging was performed through microcomputed tomography (Micro-CT). Furthermore, confocal analysis was undertaken to investigate human mesenchymal stem cell adhesion and spreading on the PCL/Fe_3O_4 nanocomposite fibers. The results suggest that nanoparticles mechanically reinforced the PCL matrix; the elastic modulus and the maximum stress increased about 10 and 30%, respectively. However, the maximum strain decreased about 50%; this suggested an enhanced brittleness. Magnetic results evidenced a superparamagnetic behavior for these nanocomposite scaffolds. Micro-CT suggested an almost uniform distribution of nanoparticles. Confocal analysis highlighted interesting results in terms of cell adhesion and spreading. All of these results show that a magnetic feature could be incorporated into a polymeric matrix that could be processed to manufacture scaffolds for advanced bone tissue engineering and, thus, provide new opportunity in terms of scaffold fixation and functionalization.

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De Santis, R., et al. "**A route toward the development of 3D magnetic scaffolds with tailored mechanical and morphological properties for hard tissue regeneration: Preliminary study.**" *Virtual and Physical Prototyping* 6.4 (2011).

A basic approach toward the design of three-dimensional (3D) rapid prototyped magnetic scaffolds for hard-tissue regeneration has been proposed. In particular, 3D scaffolds consisting of a poly(ε-caprolactone) (PCL) matrix and iron oxide (Fe3O4) or iron-doped hydroxyapatite (FeHA) nanoparticles were fabricated through a 3D fibre deposition technique. As a first approach, a polymer to nanoparticle weight ratio of 90/10 (wt/wt) was used. The effect of the inclusion of both kinds of nanoparticles on the mechanical, magnetic, and biological performances of the scaffolds was studied. The inclusion of Fe3O4 and FeHA nanoparticles generally improves the modulus and the yield stress of the fibres if compared to those of neat PCL, as well as the modulus of the scaffolds. Micro-computed tomography has confirmed the possibility to design morphologically-controlled structures with a fully interconnected pore network. Magnetisation analyses performed at 37°C have highlighted M-H curves that are not hysteretic; values of saturation magnetisation (Ms) of about 3.9 emu/g and 0.2 emu/g have been evaluated for PCL/Fe3O4 and PCL/FeHA scaffolds, respectively. Furthermore, results from confocal laser scanning microscopy (CLSM) carried out on cell-scaffold constructs have evidenced that human mesenchymal stem cells (hMSCs) better adhered and were well spread on the PCL/Fe3O4 and PCL/FeHA nanocomposite scaffolds in comparison with the PCL structures.

Feito, María José, et al. "**Immobilization and bioactivity evaluation of FGF-1 and FGF-2 on powdered silicon-doped hydroxyapatite and their scaffolds for bone tissue engineering.**" *Journal of Materials Science: Materials in Medicine* 22.2 (2011): 405-416.

Fibroblast growth factors (FGFs) are polypeptides that control the proliferation and differentiation of various cell types including osteoblasts. FGFs are also strong inducers of angiogenesis, necessary to obtain oxygen and nutrients during tissue repair. With the aim to incorporate these desirable FGF biological properties into bioceramics for bone repair, silicon substituted hydroxyapatites (Si-HA) were used as materials to immobilize bioactive FGF-1 and FGF-2. Thus, the binding of these growth factors to powdered Si-HA and Si-HA scaffolds was carried out efficiently in the present study and both FGFs maintained its biological activity on osteoblasts after its immobilization. The improvement of cell adhesion and proliferation onto Si-HA scaffolds suggests the potential utility of these FGF/scaffolds for bone tissue engineering.

García, Ana, et al. "**Preparation of 3-D scaffolds in the SiO_2–P_2O_5 system with tailored hierarchical meso-macroporosity.**" *Acta biomaterialia* 7.3 (2011): 1265-1273.

Herein we report for the first time the synthesis of three-dimensional scaffolds in the binary system SiO_2–P_2O_5 exhibiting different scales of porosity: (i) highly ordered mesopores with diameters of ca. 4 nm; (ii) macropores with diameters in the 30–80 μm range with interconnections of ca. 2–4 and 8–9 μm; and (iii) ultra-large macropores of ca. 400 μm. The hierarchical porosity of the resulting scaffolds makes them suitable for bone tissue engineering applications. The chemical nature and mesoporosity of these matrices would allow these scaffolds to act as local controlled delivery systems of biologically active molecules, such as certain drugs to treat bone pathologies. The synthetic method consists of the combination of a single-step sol–gel route in the presence of a surfactant as the mesostructure directing agent and a biomacromolecular polymer such as methylcellulose as the macrostructure template followed by rapid prototyping technique. An exhaustive study of the aging process as well as of the rheological properties of the slurry after methylcellulose addition has been carried out to obtain hierarchical meso-macroporosity. This study allows the establishment of the time period in which the slurry presents appropriate viscosity to be extruded during the rapid prototyping once the ink is prepared. The setting up of this manufacture process at the laboratory level is important from the industrial point of view when the large-scale production of scaffolds for bone tissue repair and regeneration is targeted.

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Kammerer, M., et al. "**Valproate release from polycaprolactone implants prepared by 3D-bioplotting.**" *Die Pharmazie-An International Journal of Pharmaceutical Sciences* 66.7 (2011): 511-516.

In this study we examined the release kinetics of valproate from polycaprolactone (PCL) implants constructed for local antiepileptic therapy. The PCL implants were produced with a novel 3D-Bioplotting technology. Release kinetics were determined by superfusion of these implants. Valproate was measured in the superfusate fractions with high pressure liquid chromatography (HPLC). The HPLC measurements were linear over a concentration range of 10–500 g/mL for valproate and the limit of quantification was found to be 9 g/mL. The HPLC method used is simple, accurate and sensitive. Within the first day, valproate (10%w/w)-PCL implants released already 77% of the maximum possible liberated amount whereas (5%w/w)-PCL implants released only 53%. After four days, 88% of valproate was released from (10%w/w)-PCL implants and 94% valproate from (5%w/w)-PCL implants. When valproate was ground before the 3D-Bioplotting process, only 63% from (10%w/w)-PCL implants was released within the first day. This released amount of ground valproate was significantly lower compared to that which was not ground from the (10%w/w)-PCL implants. After three days of superfusion a total amount of 89% of ground valproate within the implants was released, corresponding to 88% of non-ground valproate after four days. The fast releasing PCL implants can be used to study acute effects of locally applied valproate on epileptogenesis in vivo after initiation of an epileptic focus in an animal model. The corresponding biocompatibility may also be analysed.

Maher, Paul S., et al. "**Thermal imaging analysis of 3D biological agarose matrices.**" *International Journal of Medical Engineering and Informatics* 3.2 (2011): 167-179.

Advances in rapid prototyping have allowed for the construction of biocompatible materials (hydrogels) to be used in regenerative medicine. Within this area of construction inherent problems arise due to the mechanical instability of such materials that are temperature dependent. This research paper describes a thermal imaging analysis used to circumvent needle blockage when using an RP technology called bioplotting, used for extruding high temperature hydrogels, where agarose was the experimental biomaterial. The investigation describes how we have overcome these inherent problems through thermal imaging analysis, allowing us to accurately construct 3D biological matrices that have satisfied the in-vitro cell requirements for producing artificial tissue scaffolds. By properly insulating the needle and chamber, we have reduced the time taken for the needle to reach a sufficient plotting temperature. The analysis has allowed us to produce 3D biological matrices that have satisfied the in vitro cell requirements for producing artificial tissue. The analysis reported in this paper has opened the possibility for other high temperature dependent hydrogels to be constructed into 3D biological matrices without delay.

Manzano, Miguel, et al. "**Comparison of the osteoblastic activity conferred on Si-doped hydroxyapatite scaffolds by different osteostatin coatings.**" *Acta Biomaterialia* 7.10 (2011): 3555-3562.

Parathyroid hormone-related protein (107-111) (osteostatin) induces osteogenic effects in osteoblasts in vitro and in regenerating bone in mice and rabbits. In this study we used osteoblastic MC3T3-E1 cell cultures to evaluate and compare the bioactivity of this peptide either adsorbed or covalently bound (by its C-terminus) to Si-doped hydroxyapatite (Si-HA) scaffolds after organic (–NH2) functionalization. By these means osteostatin can be locally released or kept anchored to the scaffold surface. This was confirmed by chemical analysis and by testing the efficiency of osteostatin-loaded Si-HA scaffolds (placed in Transwell chambers) in healing a scratch wound in mouse pluripotent mesenchymal C3H10T1/2 cells. Our results show that exposure of MC3T3-E1 cell monolayers to Si-HA scaffolds with both types of osteostatin coating (deliverable or immobilized), in contrast to those without peptide, similarly stimulated cell growth and matrix mineralization. These findings demonstrate that osteostatin

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release from Si-HA scaffolds is not essential to promote osteoblastic growth and function in vitro, and lend credence to considering osteostatin a bone regenerating factor.

Schumann, Paul, et al. "**Comparably accelerated vascularization by preincorporation of aortic fragments and mesenchymal stem cells in implanted tissue engineering constructs.**" *Journal of Biomedical Materials Research Part A* 97.4 (2011): 383-394.

The demanding need for tissue replacement resulted in manifold approaches for the construction of different tissues. One common problem which hampers the clinical usage of tissue engineering constructs is a limited vascularization. In an attempt to accelerate the vascularization of tissue engineering constructs we compared the usage of bone marrow mesenchymal stem cells (bmMSCs) and fragments derived from the aorta in vivo. Tissue engineering constructs composed of PLGA scaffolds containing Matrigel (n = 8), aortic fragments embedded in Matrigel (n = 8), bmMSCs embedded in Matrigel (n = 8), and aortic fragments embedded in Matrigel combined with bmMSCs (n = 8) were implanted into dorsal skinfold chambers of balb/c mice and analyzed repetitively over 14 days. In all groups a weak inflammatory response was transiently apparent. Vascularization was significantly (p= 0.05) accelerated in bmMSC and aortic fragments containing constructs compared with Matrigel alone, demonstrated by a distinctly increased microvascular density throughout the whole experiment. The combination of bmMSCs and aortic fragments showed no additional effect compared with bmMSCs and aortic fragments alone. The accelerated vascularization and microvascular density of tissue engineering constructs triggered by bmMSCs and aortic fragments is comparable. Thus aortic fragments provide a new promising source for clinical relevant tissue engineering constructs.

Sobral, Jorge M., et al. "**Three-dimensional plotted scaffolds with controlled pore size gradients: effect of scaffold geometry on mechanical performance and cell seeding efficiency.**" *Acta Biomaterialia* 7.3 (2011): 1009-1018.

Scaffolds produced by rapid prototyping (RP) techniques have proved their value for tissue engineering applications, due to their ability to produce predetermined forms and structures featuring fully interconnected pore architectures. Nevertheless, low cell seeding efficiency and non-uniform distribution of cells remain major limitations when using such types of scaffold. This can be mainly attributed to the inadequate pore architecture of scaffolds produced by RP and the limited efficiency of cell seeding techniques normally adopted. In this study we aimed at producing scaffolds with pore size gradients to enhance cell seeding efficiency and control the spatial organization of cells within the scaffold. Scaffolds based on blends of starch with poly(ε-caprolactone) featuring both homogeneously spaced pores (based on pore sizes of 0.75 and 0.1 mm) and pore size gradients (based on pore sizes of 0.1–0.75–0.1 and 0.75–0.1–0.75 mm) were designed and produced by three-dimensional plotting. The mechanical performance of the scaffolds was characterized using dynamic mechanical analysis (DMA) and conventional compression testing under wet conditions and subsequently characterized using scanning electron microscopy and micro-computed tomography. Osteoblast-like cells were seeded onto such scaffolds to investigate cell seeding efficiency and the ability to control the zonal distribution of cells upon seeding. Scaffolds featuring continuous pore size gradients were originally produced. These scaffolds were shown to have intermediate mechanical and morphological properties compared with homogenous pore size scaffolds. The pore size gradient scaffolds improved seeding efficiency from ~35% in homogeneous scaffolds to ~70% under static culture conditions. Fluorescence images of cross-sections of the scaffolds revealed that scaffolds with pore size gradients induce a more homogeneous distribution of cells within the scaffold.

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Tavassol, Frank, et al. "**A novel approach for studying microcirculation in bone defects by intravital fluorescence microscopy.**" *Tissue Engineering Part C: Methods* 17.12 (2011): 1151-1159.

Angiogenic and inflammatory responses to biodegradable scaffolds were previously studied using the dorsal skinfold chamber for testing different scaffold materials. In this model, the angiogenic response originates from the soft tissue of the skin. Herein, we introduce a new model that allows the study of developing microcirculation of bone defects for testing tissue-engineered constructs. A bone defect was prepared in the femur of Balb/c mice by inserting a pin for intramedullary fixation, and a custom-made observation window fixed over the defect allowed constant observation. This study included three different groups: empty defect (control), defect filled with porous poly(l-lactide-co-glycolide), and beta-tricalcium-phosphate scaffolds. Starting from 6 days after surgery, angiogenesis, neovascularization, leukocyte–endothelial cell interaction, and microvascular permeability were analyzed over 22 days by using intravital fluorescence microscopy. The empty defects showed no signs of angiogenesis during the observation period, but a distinct increase of capillary density was detected in the scaffold-containing defects. Surprisingly, the histological sections of the scaffold-treated defects showed new bone formation 22 days after implantation. We present a new bone chamber model for intravital long-term study of scaffold materials suitable for bone reconstruction in mice by using fluorescence microscopy.

Tavassol, Frank, et al. "**Prolongated survival of osteoblast-like cells on biodegradable scaffolds by heat shock preconditioning.**" *Tissue Engineering Part A* 17.15-16 (2011): 1935-1943.

The implantation of tissue-engineered constructs leads to hypoxic and physical stress to the seeded cells until they were reached by a functional microvascular system. Preconditioning of cells with heat shock induced heat shock proteins, which can support the cells to survive a subsequent episode of stress that would otherwise be lethal. Preconditioning of tissue-engineered constructs resulted in significantly higher number of surviving osteoblast-like cells (OLC). At the 6th and 10th day, angiogenic response was found comparative to poly(L-lactide-co-glycolide) (PLGA) scaffolds vitalized with either unconditioned or preconditioned OLC. However, they were significantly enhanced compared with the nonvitalized collagen-labeled PLGA scaffolds. This study demonstrates that vitalization of PLGA scaffolds with OLC accelerates the angiogenic response induced by the surrounding host tissue. In addition, heat shock preconditioning significantly enhances the survival rate of the OLC that are seeded on these scaffolds. Thus, vitalization of substitutes with adequately pretreated OLC may promise biologically adequate osseous restorations.

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Chien, Karen B., Emmanuella Makridakis, and Ramille N. Shah. "**Three-dimensional printing of soy protein scaffolds for tissue regeneration.**" *Tissue Engineering Part C: Methods* 19.6 (2012): 417-426.

Fabricating three-dimensional (3D) porous scaffolds with controlled structure and geometry is crucial for tissue regeneration. To date, exploration in printing 3D natural protein scaffolds is limited. In this study, soy protein slurry was successfully printed using the 3D Bioplotter to form scaffolds. A method to verify the structural integrity of resulting scaffolds during printing was developed. This process involved measuring the mass extrusion flow rate of the slurry from the instrument, which was directly affected by the extrusion pressure and the soy protein slurry properties. The optimal mass flow rate for printing soy slurry at 27°C was 0.0072±0.0002 g/s. The addition of dithiothreitol to soy slurries demonstrated the importance of disulfide bonds in forming solid structures upon printing. Resulting Bioplotted soy protein scaffolds were cured using 95% ethanol and post-treated using dehydrothermal treatment (DHT), a combination of freeze-drying and DHT, and chemical crosslinking using 1-ethyl-3-(3 dimethylaminopropyl)carbodiimide (EDC) chemistry. Surface morphologies of the different treatment groups were characterized using scanning electron microscopy. Scaffold properties, including relative crosslink density, mass loss upon rinsing, and compressive modulus revealed that EDC crosslinked scaffolds were the most robust with moduli of approximately 4 kPa. Scaffold geometry (45° and 90° layer rotations) affected the mechanical properties for DHT and EDC crosslinked scaffolds. Seeding efficiency of human mesenchymal stem cells (hMSC) was highest for nontreated and thermally treated scaffolds, and all scaffolds supported hMSC viability over time.

Daoud, Jamal, et al. "**Dielectric spectroscopy for non-invasive monitoring of epithelial cell differentiation within three-dimensional scaffolds.**" *Physics in Medicine and Biology* 57.16 (2012): 5097.

In this study, we introduce a cellular differentiation cellular model based on dielectric spectroscopy that characterizes epithelial differentiation processes. Non-invasive cellular monitoring was achieved within a three-dimensional microenvironment consisting of a cell-containing collagen I gel seeded onto microfabricated scaffolds. In this proof-of-concept investigation, Madin–Darby canine kidney cells were cultured within microfabricated, geometrically controlled scaffolds and allowed us to differentiate to hollow cyst-like structures. This transformation within the three-dimensional environment is monitored and characterized through dielectric spectroscopy while maintaining cell culture in vitro.

Gloria, A., et al. "**Three-dimensional poly (ε-caprolactone) bioactive scaffolds with controlled structural and surface properties.**" *Biomacromolecules* 13.11 (2012): 3510-3521.

The requirement of a multifunctional scaffold for tissue engineering capable to offer at the same time tunable structural properties and bioactive interface is still unpaired. Here we present three-dimensional (3D) biodegradable polymeric (PCL) scaffolds with controlled morphology, macro-, micro-, and nano-mechanical performances endowed with bioactive moieties (RGD peptides) at the surface. Such result was obtained by a combination of rapid prototyping (e.g., 3D fiber deposition) and surface treatment approach (aminolysis followed by peptide coupling). By properly designing process conditions, a control over the mechanical and biological performances of the structure was achieved with a capability to tune the value of compressive modulus (in the range of 60–90 MPa, depending on the specific lay-down pattern). The macromechanical behavior of the proposed scaffolds was not affected by surface treatment preserving bulk properties, while a reduction of hardness from 0.50–0.27 GPa to 0.1–0.03 GPa was obtained. The penetration depth of the chemical treatment was determined by nanoindentation measurements and confocal microscopy. The efficacy of both functionalization and the following bioactivation was monitored by analytically quantifying functional groups and/or peptides at the interface. NIH3T3 fibroblast adhesion studies evidenced that cell attachment was improved, suggesting a correct presentation of the peptide. Accordingly, the present work mainly focuses on the effect of the surface modification on the mechanical and functional performances of the scaffolds, also

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showing a morphological and analytical approach to study the functionalization/bioactivation treatment, the distribution of immobilized ligands, and the biological features.

Oliveira, A. L., et al. "**Peripheral mineralization of a 3D biodegradable tubular construct as a way to enhance guidance stabilization in spinal cord injury regeneration.**" *Journal of Materials Science: Materials in Medicine* 23.11 (2012): 2821-2830.

Spinal cord injuries (SCI) present a major challenge to therapeutic development due to its complexity. Combinatorial approaches using biodegradable polymers that can simultaneously provide a tissue scaffold, a cell vehicle, and a reservoir for sustained drug delivery have shown very promising results. In our previous studies we have developed a novel hybrid system consisting of starch/poly-e-caprolactone (SPCL) semi-rigid tubular porous structure, based on a rapid prototyping technology, filled by a gellan gum hydrogel concentric core for the regeneration within spinal-cord injury sites. In the present work we intend to promote enhanced osteointegration on these systems by pre-mineralizing specifically the external surfaces of the SPCL tubular structures, though a biomimetic strategy, using a sodium silicate gel as nucleating agent. The idea is to create two different cell environments to promote axonal regeneration in the interior of the constructs while inducing osteogenic activity on its external surface. By using a Teflon cylinder to isolate the interior of the scaffold, it was possible to observe the formation of a bone-like poorly crystalline carbonated apatite layer continuously formed only in the external side of the tubular structure. This biomimetic layer was able to support the adhesion of Bone Marrow Mesenchymal Stem Cells, which have gone under cytoskeleton reorganization in the first hours of culture when compared to cells cultured on uncoated scaffolds. This strategy can be a useful route for locally stimulate bone tissue regeneration and facilitating early bone ingrowth.

Silva, Nuno A., et al. "**Benefits of spine stabilization with biodegradable scaffolds in spinal cord injured rats.**" *Tissue Engineering Part C: Methods* 19.2 (2012): 101-108.

Spine stabilization upon spinal cord injury (SCI) is a standard procedure in clinical practice, but rarely employed in experimental models. Moreover, the application of biodegradable biomaterials for this would come as an advantage as it would eliminate the presence of a nondegradable prosthesis within the vertebral bone. Therefore, in the present work, we propose the use of a new biodegradable device specifically developed for spine stabilization in a rat model of SCI. A 3D scaffold based on a blend of starch with polycaprolactone was implanted, replacing delaminated vertebra, in male Wistar rats with a T8-T9 spinal hemisection. The impact of spinal stabilization on the locomotor behavior was then evaluated for a period of 12 weeks. Locomotor evaluation—assessed by Basso, Beatie, and Bresnahan test; rotarod; and open field analysis—revealed that injured rats subjected to spine stabilization significantly improved their motor performance, including higher coordination and rearing activity when compared with SCI rats without stabilization. Histological analysis further revealed that the presence of the scaffolds not only stabilized the area, but also simultaneously prevented the infiltration of the injury site by connective tissue. Overall, these results reveal that SCI stabilization using a biodegradable scaffold at the vertebral bone level leads to an improvement of the motor deficits and is a relevant element for the successful treatment of SCI.

Silva, Nuno A., et al. "**Interactions between Schwann and olfactory ensheathing cells with a starch/polycaprolactone scaffold aimed at spinal cord injury repair.**" *Journal of Biomedical Materials Research Part A* 100.2 (2012): 470-476.

Spinal cord injury (SCI) represents a major world health problem. Therefore it is urgent to develop novel strategies that can specifically target it. We have previously shown that the implantation of starch-based scaffolds (SPCL) aimed for spine stabilization on SCI animals leads to motor skills improvements. Therefore, we hypothesize that the combination of these scaffolds with relevant cell populations for SCI repair will, most likely, lead to further improvements. Therefore, in this work, the ability of SPCL scaffolds to support the 3D culture of olfactory ensheathing cells (OECs) and Schwann cells (SCs) was

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studied and characterized. The results demonstrate for the first time that SPCL scaffolds were able to support the growth and migration of OECs and SCs. Moreover, the results indicate that two weeks of in vitro culture is the ideal time to reach a high number of transplantable cells. Future work will focus on the spine stabilization of SCI animals using SPCL scaffolds loaded with OECs or SCs for SCI regeneration.

Sun, Yang, et al. "**Degradable amorphous scaffolds with enhanced mechanical properties and homogeneous cell distribution produced by a three-dimensional fiber deposition method.**" *Journal of Biomedical Materials Research Part A* 100.10 (2012): 2739-2749.

The mechanical properties of amorphous, degradable, and highly porous poly(lactide-co-caprolactone) structures have been improved by using a 3D fiber deposition (3DF) method. Two designs of 3DF scaffolds, with 45° and 90° layer rotation, were printed and compared with scaffolds produced by a salt-leaching method. The scaffolds had a porosity range from 64% to 82% and a high interconnectivity, measured by micro-computer tomography. The 3DF scaffolds had 8–9 times higher compressive stiffness and 3–5 times higher tensile stiffness than the salt-leached scaffolds. There was a distinct decrease in the molecular weight during printing as a consequence of the high temperature. The chain microstructure was, however, not affected; the glass transition temperature and the decomposition temperature were constant. Human OsteoBlast-like cells were cultured in vitro and the cell morphology and distribution were observed by scanning electron microscopy and fluorescence microscopy. The cell distribution on the 3DF scaffolds was more homogeneous than the salt-leached scaffolds, suggesting that 3DF scaffolds are more suitable as porous biomaterials for tissue engineering. These results show that it is possible to design and optimize the properties of amorphous polymer scaffolds. The 3DF method produce amorphous degradable poly(lactide-co-caprolactone) that are strong and particularly suitable for cell proliferation.

Tölle, Folke Johannes, Martin Fabritius, and Rolf Mülhaupt. "**Emulsifier-Free Graphene Dispersions with High Graphene Content for Printed Electronics and Freestanding Graphene Films.**" *Advanced Functional Materials* 22.6 (2012): 1136-1144.

A novel and highly versatile synthetic route for the production of functionalized graphene dispersions in water, acetone, and isopropanol (IPA), which exhibit long-term stability and are easy to scale up, is reported. Both graphene functionalization (wherein the oxygen content can be varied from 4 to 16 wt%) and dispersion are achieved by the thermal reduction of graphite oxide, followed by a high-pressure homogenization (HPH) process. For the first time, binders, dispersing agents, and reducing agents are not required to produce either dilute or highly concentrated dispersions of single graphene sheets with a graphene content of up to 15 g L−1. High graphene content is essential for the successful printing of graphene dispersions by 3D microextrusion. Free-standing graphene films and micropatterned graphene materials are successfully prepared using this method. Due to the absence of toxic reducing agents, the graphene exhibits no cytotoxicity and is biocompatible. Furthermore, the electrical conductivity of graphene is significantly improved by the absence of binders. Flexible microarrays can be printed on different substrates, producing microarrays that are mechanically stable and can be bent several times without affecting electrical conductivity.

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Amorosa, L. F., et al. "**Physiologic load-bearing characteristics of autografts, allografts, and polymer-based scaffolds in a critical sized segmental defect of long bone: an experimental study.**" *International Journal of Nanomedicine* 8 (2013): 1637.

Background: To address the challenge of treating critical sized intercalary defects, we hypothesized that under physiologic cyclic loading, autografts, allografts, and scaffolds loaded with and without human mesenchymal stem cells (hMSCs) would have different biomechanical characteristics.
Methods: Using a rat femoral defect model, 46 rats were assigned to four groups, ie, autograft (n = 12), allograft (n = 10), scaffold (n = 13), and scaffold with hMSCs (n = 11). The scaffold groups used a 5 mm segment of scaffold composed of 80% poly-ε-caprolactone and 20% hydroxyapatite. Rats were sacrificed 4 months postoperatively, and the repairs were assessed radiographically and biomechanically.
Results: Autograft and allograft groups exhibited the most bridging callus, while the scaffold/hMSCs group had more callus than the scaffold repairs. Although signs of radiographic healing did not accurately reflect restoration of mechanical properties, addition of hMSCs on the scaffold enhanced bone formation. The scaffold alone group had significantly lower elastic and viscous stiffness and higher phase angles than other repairs and the contralateral controls. Addition of hMSCs increased the elastic and viscous stiffness of the repair, while decreasing the phase angle.
Conclusion: Further comparative analysis is needed to optimize clinical use of scaffolds and hMSCs for critical sized defect repairs. However, our results suggest that addition of hMSCs to scaffolds enhances mechanical simulation of native host bone.

Angarano, Marco, et al. "**Layered Gradient Nonwovens of In Situ Crosslinked Electrospun Collagenous Nanofibers Used as Modular Scaffold Systems for Soft Tissue Regeneration.**" *Advanced Functional Materials* 23.26 (2013): 3277-3285.

In a versatile modular scaffold system, gradient nonwovens of in situ crosslinked gelatin nanofibers (CGN), fabricated by reactive electrospinning, are laminated with perforated layers and nonwovens of thermoplastic non-crosslinked biodegradable polyesters. The addition of glyoxal to a gelatin solution in a non-toxic solvent mixture consisting of acetic acid, ethyl acetate, and water (5:3:2 w/w/w) enables the in situ crosslinking of gelatin nanofibers during electrospinning. The use of this fluorine-free crosslinking system eliminates the need of post-treatment crosslinking and purification steps typical for conventional CGN scaffolds. The slowly progressing crosslinking of the dissolved gelatin in the presence of glyoxal increases the viscosity of the gelatin solution during electrospinning so that the average diameter of the crosslinked gelatin nanofibers gradually increases from 90 to 680 nm. During the subsequent lamination process, alternating layers of CGN and polycaprolactone (PCL) nonwovens, produced by 3D microextrusion of micrometer-sized PCL fibers, are bonded together upon heating above the PCL melting temperature. In contrast to the water-soluble gelatin nanofibers and the comparatively weak CGN, the CGN/PCL/CGN layered biocomposites are water-resistant and very robust. In such modular scaffold systems, strength, biodegradation rate, and biological functions can be controlled by varying the type, composition, fiber diameter, porosity, number, and sequence of the individual layers. The CGN/PCL multilayer biocomposites can be cut into any desired scaffold shape and attached to tissue by surgical sutures in order to suit the needs of individual patients.

Bettahalli, N. M. S., et al. "**Corrugated round fibers to improve cell adhesion and proliferation in tissue engineering scaffolds.**" *Acta Biomaterialia* 9.6 (2013): 6928-6935.

Optimal cell interaction with biomaterial scaffolds is one of the important requirements for the development of successful in vitro tissue-engineered tissues. Fast, efficient and spatially uniform cell adhesion can improve the clinical potential of engineered tissue. Three-dimensional (3-D) solid free form fabrication is one widely used scaffold fabrication technique today. By means of deposition of polymer fibers, scaffolds with various porosity, 3-D architecture and mechanical properties can be prepared. These scaffolds consist mostly of solid round fibers. In this study, it was hypothesized that a corrugated fiber morphology enhances cell adhesion and proliferation and therefore leads to the development of

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successful in vitro tissue-engineered constructs. Corrugated round fibers were prepared and characterized by extruding poly(ethylene oxide terephthalate)-co-poly(butylene terephthalate) (300PEOT55PBT45) block co-polymer through specially designed silicon wafer inserts. Corrugated round fibers with 6 and 10 grooves on the fiber surface were compared with solid round fibers of various diameters. The culture of mouse pre-myoblast (C2C12) cells on all fibers was studied under static and dynamic conditions by means of scanning electron microscopy, cell staining and DNA quantification. After 7 days of culturing under static conditions, the DNA content on the corrugated round fibers was approximately twice as high as that on the solid round fibers. Moreover, under dynamic culture conditions, the cells on the corrugated round fibers seemed to experience lower mechanical forces and therefore adhered better than on the solid round fibers. The results of this study show that the surface architecture of fibers in a tissue engineering scaffold can be used as a tool to improve the performance of the scaffold in terms of cell adhesion and proliferation.

Chien, Karen B., et al. "**In vivo acute and humoral response to three-dimensional porous soy protein scaffolds.**" *Acta Biomaterialia* 9.11 (2013): 8983-8990.

Increasing interest in using soy biomaterials for tissue engineering applications has prompted investigation into the in vivo biocompatibility of soy implants. In this study, the biocompatibility of soy protein scaffolds fabricated using freeze-drying and 3-D printing was assessed using a subcutaneous implant model in BALB/c mice. The main objectives of this study were: (1) to compare soy protein with bovine collagen, a well-characterized natural protein implant, by implanting scaffolds of the same protein weight, and (2) to observe the effects of soy scaffold microstructure and amount of protein loading, which also alters the degradation properties, on the acute and humoral immune responses towards soy. Results showed that freeze-dried soy scaffolds fully degraded after 14 days, whereas collagen scaffolds (of the same protein weight) remained intact for 56 days. Furthermore, Masson's trichrome staining showed little evidence of damage or fibrosis at the soy implant site. Scaffolds of higher soy protein content, however, were still present after 56 days. H&E staining revealed that macrophage infiltration was hindered in the denser bioplotted soy scaffolds, causing slower degradation. Analysis of soy-specific antibodies in mouse serum after implantation revealed levels of IgG1 that correlated with higher scaffold weight and protein density. However, no soy-specific IgE was detected, indicating the absence of an allergic response to the soy implants. These results demonstrate that soy protein could be an acceptable biocompatible implant for tissue regeneration, and that scaffold porosity, soy protein density and scaffold degradation rate significantly affect the acute and humoral immune response.

Chung, Eun Ji, Adam E. Jakus, and Ramille N. Shah. "**In situ forming collagen–hyaluronic acid membrane structures: mechanism of self-assembly and applications in regenerative medicine.**" *Acta Biomaterialia* 9.2 (2013): 5153-5161.

Bioactive, in situ forming materials have the potential to complement minimally invasive surgical procedures and enhance tissue healing. For such biomaterials to be adopted in the clinic, they must be cost-effective, easily handled by the surgeon and have a history of biocompatibility. To this end, we report a novel and facile self-assembling strategy to create membranes and encapsulating structures using collagen and hyaluronic acid (HA). Unlike membranes built by layer-by-layer deposition of oppositely charged biomolecules, the collagen–HA membranes described here form a diffusion barrier upon electrostatic interaction of the oppositely charged biomolecules, which is further driven by osmotic pressure imbalances. The resulting membranes have a nanofibrous architecture, a thicknesses of 130 μm and a tensile modulus (0.59 ± 0.06 MPa) that can increase 7-fold using carbodiimide chemistry (4.42 ± 1.46 MPa). Collagen–HA membranes support mesenchymal stem cell proliferation and have a slow and steady protein release profile (7% at day 28), offering opportunities for targeted tissue regeneration. We demonstrate the capacity to encapsulate cells by injecting HA into the collagen solution, and enhance allograft and implant biocompatibility through a coating technique. This study describes a novel mechanism of collagen–HA membrane formation and provides the groundwork to apply these membranes in a variety of tissue engineering applications.

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Hendriks, J. A. A., et al. "**The effect of scaffold-cell entrapment capacity and physico-chemical properties on cartilage regeneration.**" *Biomaterials* 34.17 (2013): 4259-4265.

An important tenet in designing scaffolds for regenerative medicine consists in mimicking the dynamic mechanical properties of the tissues to be replaced to facilitate patient rehabilitation and restore daily activities. In addition, it is important to determine the contribution of the forming tissue to the mechanical properties of the scaffold during culture to optimize the pore network architecture. Depending on the biomaterial and scaffold fabrication technology, matching the scaffolds mechanical properties to articular cartilage can compromise the porosity, which hampers tissue formation. Here, we show that scaffolds with controlled and interconnected pore volume and matching articular cartilage dynamic mechanical properties, are indeed effective to support tissue regeneration by co-cultured primary and expanded chondrocyte (1:4). Cells were cultured on scaffolds in vitro for 4 weeks. A higher amount of cartilage specific matrix (ECM) was formed on mechanically matching (M) scaffolds after 28 days. A less protein adhesive composition supported chondrocytes rounded morphology, which contributed to cartilaginous differentiation. Interestingly, the dynamic stiffness of matching constructs remained approximately at the same value after culture, suggesting a comparable kinetics of tissue formation and scaffold degradation. Cartilage regeneration in matching scaffolds was confirmed subcutaneously in vivo. These results imply that mechanically matching scaffolds with appropriate physico-chemical properties support chondrocyte differentiation.

Higuera Sierra, G. A., et al. "**In vivo screening of extracellular matrix components produced under multiple experimental conditions implanted in one animal.**" *Integrative biology* 2013.6 (2013): 889-898.

Animal experiments help to progress and ensure safety of an increasing number of novel therapies, drug development and chemicals. Unfortunately, these also lead to major ethical concerns, costs and limited experimental capacity. We foresee a coercion of all these issues by implantation of well systems directly into vertebrate animals. Here, we used rapid prototyping to create wells with biomaterials to create a three-dimensional (3D) well-system that can be used in vitro and in vivo. First, the well sizes and numbers were adjusted for 3D cell culture and in vitro screening of molecules. Then, the functionality of the wells was evaluated in vivo under 36 conditions for tissue regeneration involving human mesenchymal stem cells (hMSCs) and bovine primary chondrocytes (bPCs) screened in one animal. Each biocompatible well was controlled to contain µl-size volumes of tissue, which led to tissue penetration from the host and tissue formation under implanted conditions. We quantified both physically and biologically the amounts of extracellular matrix (ECM) components found in each well. Using this new concept the co-culture of hMSCs and bPCs was identified as a positive hit for cartilage tissue repair, which was a comparable result using conventional methods. The in vivo screening of candidate conditions opens an entirely new range of experimental possibilities, which significantly abates experimental animal use and increases the pace of discovery of medical treatments.

Huri, Pinar Yilgor, et al. "**A biomimetic growth factor delivery strategy for enhanced regeneration of iliac crest defects.**" *Biomedical Materials* 8.4 (2013): 045009.

The importance of provision of growth factors in the engineering of tissues has long been shown to control the behavior of the cells within the construct and several approaches were applied toward this end. In nature, more than one type of growth factor is known to be effective during the healing of tissue defects and their peak concentrations are not always simultaneous. One of the most recent strategies includes the delivery of a combination of growth factors with the dose and timing to mimic the natural regeneration cascade. The sequential delivery of bone morphogenetic proteins BMP-2 and BMP-7 which are early and late appearing factors during bone regeneration, respectively, was shown in vitro to enhance osteoblastic differentiation of bone marrow derived mesenchymal stem cells. In the present study, the aim was to study the effectiveness of this delivery strategy in a rabbit iliac crest model. 3D

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plotted poly(ε-caprolactone) scaffolds were loaded with BMP carrying nanoparticles to achieve: (a) single BMP-2 or BMP-7 delivery, and (b) their combined delivery in a simultaneous or (c) sequential (biomimetic) fashion. After eight weeks of implantation, computed tomography and biomechanical tests showed better mineralized matrix formation and bone-implant union strength at the defect site in the case of sequential delivery compared to single or simultaneous delivery modes. Bone mineral density (BMD) and push-out stress were: 33.65±2.25 g cm^{-3} and 14.5±2.28 MPa, respectively, and almost 2.5 fold higher in comparison to those without growth factors (BMD: 14.14±1.21 g cm^{-3}; PS: 6.59±0.65 MPa). This study, therefore, supports those obtained in vitro and emphasizes the importance of mimicking the natural timing of bioavailability of osteogenic factors in improving the regeneration of critical-sized bone defects.

Izadifar, Zohreh, Leroy Dean Chapman, and Xiongbiao Chen. "**Computed Tomography Diffraction-Enhanced Imaging for In Situ Visualization of Tissue Scaffolds Implanted in Cartilage.**" *Tissue Engineering Part C: Methods* 20.2 (2013): 140-148.

Long-term in vivo studies on animal models and advances from animal to human studies should rely on noninvasive monitoring methods. Synchrotron radiation (SR)-diffraction enhanced imaging (DEI) has shown great promise as a noninvasive method for visualizing native and/or engineered tissues and bio-microstructures with appreciable details in situ. The objective of this study was to investigate SR-DEI for in situ visualization and characterization of tissue-engineered scaffolds implanted in cartilage. A piglet stifle joint implanted with an engineered scaffold made from poly-ε-caprolactone was imaged using SR computed tomography (CT)-DEI at an X-ray energy of 40 keV. For comparison, in situ visualization was also conducted with commonly used SR CT-phase contrast imaging and clinical magnetic resonance imaging techniques. The reconstructed CT-DE images show the implanted scaffold with the structural properties much clearer than those in the CT-PC and MR images. Furthermore, CT-DEI was able to visualize microstructures within the cartilage as well as different soft tissues surrounding the joint. These microstructural details were not recognizable using other imaging techniques. Taken together, the results of this study suggest that CT-DEI can be used for noninvasive visualization and characterization of scaffolds in cartilage, representing an advance in tissue engineering to track the success of tissue scaffolds for cartilage repair.

Kampmann, Andreas, et al. "**Additive effect of mesenchymal stem cells and VEGF to vascularization of PLGA scaffolds.**" *Microvascular research* 90 (2013): 71-79.

Bone marrow derived mesenchymal stem cells (bmMSCs) are widely used for the generation of tissue engineering constructs, since they can differentiate into different cell types occurring in bone tissues. Until now their use for the generation of tissue engineering constructs is limited. All cells inside a tissue engineering construct die within a short period of time after implantation of the construct because vascularization and establishment of connections to the recipient circulatory system is a time consuming process. We therefore compared the influences of bmMSC, VEGF and a combination of both on the early processes of vascularization, utilizing the mice skinfold chamber model and intravital fluorescence microscopy.

Tissue engineering constructs based on collagen coated Poly d,l-lactide-co-glycolide (PLGA) scaffolds, were either functionalized by coating with vascular endothelial growth factor (VEGF) or vitalized with bmMSC. PLGA without cells and growth factor was used as the control group. Functionalized and vitalized tissue engineering constructs showed an accelerated growth of microvessels compared to controls. Only marginal differences in vascular growth were detected between VEGF containing and bmMSC containing constructs. Constructs containing VEGF and bmMSC showed a further enhanced microvascular growth at day 14.

We conclude that bmMSCs are well suited for bone tissue engineering applications, since they are a valuable source of angiogenic growth factors and are able to differentiate into the tissue specific cell types of interest. The dynamic process of vascularization triggered by growth factor producing cells can be amplified and stabilized with the addition of accessory growth factors, leading to a persisting

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angiogenesis, but strategies are needed that enhance the resistance of bmMSC to hypoxia and increase survival of these cells until the tissue engineering construct has build up a functional vascular system.

Kiziltay, Aysel, et al. "**Poly (ester-urethane) scaffolds: effect of structure on properties and osteogenic activity of stem cells.**" *Journal of tissue engineering and regenerative medicine* (2013).

The present study aimed to investigate the effect of structure (design and porosity) on the matrix stiffness and osteogenic activity of stem cells cultured on poly(ester-urethane) (PEU) scaffolds. Different three-dimensional (3D) forms of scaffold were prepared from lysine-based PEU using traditional salt-leaching and advanced bioplotting techniques. The resulting scaffolds were characterized by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), mercury porosimetry and mechanical testing. The scaffolds had various pore sizes with different designs, and all were thermally stable up to 300 °C. In vitro tests, carried out using rat bone marrow stem cells (BMSCs) for bone tissue engineering, demonstrated better viability and higher cell proliferation on bioplotted scaffolds compared to salt-leached ones, most probably due to their larger and interconnected pores and stiffer nature, as shown by higher compressive moduli, which were measured by compression testing. Similarly, SEM, von Kossa staining and EDX analyses indicated higher amounts of calcium deposition on bioplotted scaffolds during cell culture. It was concluded that the design with larger interconnected porosity and stiffness has an effect on the osteogenic activity of the stem cells.

Meseguer-Olmo, Luis, et al. "**In-vivo behavior of Si-hydroxyapatite/polycaprolactone/DMB scaffolds fabricated by 3D printing.**" *Journal of Biomedical Materials Research Part A* 101.7 (2013): 2038-2048.

Scaffolds made of polycaprolactone and nanocrystalline silicon-substituted hydroxyapatite have been fabricated by 3D printing rapid prototyping technique. To asses that the scaffolds fulfill the requirements to be considered for bone grafting applications, they were implanted in New Zealand rabbits. Histological and radiological studies have demonstrated that the scaffolds implanted in bone exhibited an excellent osteointegration without the interposition of fibrous tissue between bone and implants and without immune response after 4 months of implantation. In addition, we have evaluated the possibility of improving the scaffolds efficiency by incorporating demineralized bone matrix during the preparation by 3D printing. When demineralized bone matrix (DBM) is incorporated, the efficacy of the scaffolds is enhanced, as new bone formation occurs not only in the peripheral portions of the scaffolds but also within its pores after 4 months of implantation. This enhanced performance can be explained in terms of the osteoinductive properties of the DBM in the scaffolds, which have been assessed through the new bone tissue formation when the scaffolds are ectopically implanted.

Nandakumar, Anandkumar, et al. "**Combining technologies to create bioactive hybrid scaffolds for bone tissue engineering.**" *Biomatter* 3.2 (2013).

Combining technologies to engineer scaffolds that can offer physical and chemical cues to cells is an attractive approach in tissue engineering and regenerative medicine. In this study, we have fabricated polymer-ceramic hybrid scaffolds for bone regeneration by combining rapid prototyping (RP), electrospinning (ESP) and a biomimetic coating method in order to provide mechanical support and a physico-chemical environment mimicking both the organic and inorganic phases of bone extracellular matrix (ECM). Poly(ethylene oxide terephthalate)-poly(buthylene terephthalate) (PEOT/PBT) block copolymer was used to produce three dimensional scaffolds by combining 3D fiber (3DF) deposition, and ESP, and these constructs were then coated with a Ca-P layer in a simulated physiological solution. Scaffold morphology and composition were studied using scanning electron microscopy (SEM) coupled to energy dispersive X-ray analyzer (EDX) and Fourier Tranform Infrared Spectroscopy (FTIR). Bone marrow derived human mesenchymal stromal cells (hMSCs) were cultured on coated and uncoated 3DF and 3DF + ESP scaffolds for up to 21 d in basic and mineralization medium and cell attachment,

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proliferation, and expression of genes related to osteogenesis were assessed. Cells attached, proliferated and secreted ECM on all the scaffolds. There were no significant differences in metabolic activity among the different groups on days 7 and 21. Coated 3DF scaffolds showed a significantly higher DNA amount in basic medium at 21 d compared with the coated 3DF + ESP scaffolds, whereas in mineralization medium, the presence of coating in 3DF+ESP scaffolds led to a significant decrease in the amount of DNA. An effect of combining different scaffolding technologies and material types on expression of a number of osteogenic markers (cbfa1, BMP-2, OP, OC and ON) was observed, suggesting the potential use of this approach in bone tissue engineering.

Nandakumar, Anandkumar, et al. "**Monolithic and assembled polymer–ceramic composites for bone regeneration.**" *Acta Biomaterialia* 9.3 (2013): 5708-5717.

The rationale for the use of polymer–ceramic composites for bone regeneration stems from the natural composition of bone, with collagen type I and biological apatite as the main organic and inorganic constituents, respectively. In the present study composite materials of PolyActive™ (PA), a poly(ethylene oxide terephthalate)/poly(butylene terephtalate) co-polymer, and hydroxyapatite (HA) at a weight ratio of 85:15 were prepared by rapid prototyping (RP) using two routes. In the first approach pre-extruded composite filaments of PA–HA were processed using three-dimensional fibre deposition (3DF) (conventional composite scaffolds). In the second approach PA scaffolds were fabricated using 3DF and combined with HA pillars produced inside stereolithographic moulds that fitted inside the pores of the PA three-dimensional structure (assembled composite scaffolds). Analysis of calcium and phosphate release in a simulated physiological solution, not containing calcium or phosphate, revealed significantly higher values for the HA pillars compared with other scaffolds. Release in simulated body fluid saturated with respect to HA did not show significant differences among the different scaffolds. Human mesenchymal stromal cells were cultured on polymer (3DF), conventional composite (3DF-HA) and assembled composite (HA assembled in 3DF) scaffolds and assessed for morphology, metabolic activity, DNA amount and gene expression of osteogenic markers using real time quantitative polymerase chain reaction (PCR). Scanning electron microscopy images showed that the cells attached to and infiltrated all the scaffolds. Assembled composites had a higher metabolic activity compared with 3DF-HA scaffolds while no significant differences were observed in DNA amounts. Gene expression of osteopontin in the assembled composite was significantly higher compared with the conventional composites. The strategy of composite fabrication by assembly appears to be a promising alternative to the conventional composite fabrication route for scaffolds for bone regeneration.

Oliveira, Sara M., et al. "**Hierarchical Fibrillar Scaffolds Obtained by Non-conventional Layer-By-Layer Electrostatic Self-Assembly**." *Advanced healthcare materials* 2.3 (2013): 422-427.

A new application of layer-by-layer assembly is presented, able to create nano/micro fibrils or nanocoatings inside 3D scaffolds using non-fibrillar polyelectrolytes for tissue-engineering applications. This approach shows promise for developing advanced scaffolds with controlled nano/micro environments, and nature and architectures similar to the natural extracellular matrix, leading to improved biological performance.

Ragaert, Kim, et al. "**Design and thermoregulation of a new microextrusion dispense head for 3D-plotting of thermally sensitive thermoplastics.**" *Polymer Engineering & Science* 53.2 (2013): 273-282.

Three-dimensional plotting of micro-extruded filaments is a promising method for the manufacture of porous structures in thermoplastic polymers. However, it has been noted that conventional dispense head systems for this microextrusion are unsuitable for the reliable processing of thermally sensitive polymers like poly(lactic acid) (PLA): the batch polymer remains in the molten state prior to processing and it will degrade under this thermal load, leading to polymer chain reduction. Therefore, a new

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dispense head system was developed which thermally separates a cold material supply zone from the hot extrusion zone and allows for the processing of PLA-based polymers without severe degradation. This manuscript discusses the design of the new dispense head and compares its thermoregulation with that of a conventional system by means of finite element modeling and infrared imaging.

Russo, Teresa, et al. "**Systematic analysis of injectable materials and 3D rapid prototyped magnetic scaffolds: from CNS applications to soft and hard tissue repair/regeneration.**" *Procedia Engineering* 59 (2013): 233-239.

Over the past years, polymer-based materials have attracted research interest in the field of tissue repair and regeneration. As reported in literature, different injectable systems have been proposed, trying to reduce surgical invasiveness. In a first step of the current research, the rheological and functional features of injecatble hydrogel-based materials for central nervous system applications or soft tissue regeneration (collagen/PEG semi-IPNs) as well as for hard tissue engineering (alginate/iron-doped hydroxyapatite) were evaluated. Then, the study was also devoted to the development of 3D nanocomposite poly(ε- caprolactone)/iron-doped hydroxyapatite scaffolds for bone tissue engineering, providing a preliminary approach to assess magnetic attraction forces.

Samberg, Meghan E., et al. "**Biocompatibility analysis of an electrically-activated silver-based antibacterial surface system for medical device applications.**" *Journal of Materials Science: Materials in Medicine* 24.3 (2013): 755-760.

The costs associated with the treatment of medical device and surgical site infections are a major cause of concern in the global healthcare system. To prevent transmission of such infections, a prophylactic surface system that provides protracted release of antibacterial silver ions using low intensity direct electric current (LIDC; 28 μA system current at 6 V) activation has been recently developed. To ensure the safety for future in vivo studies and potential clinical applications, this study assessed the biocompatibility of the LIDC-activated interdigitated silver electrodes-based surface system; in vitro toxicity to human epidermal keratinocytes, human dermal fibroblasts, and normal human osteoblasts, and antibacterial efficacy against Staphylococcus aureus and Escherichia coli was evaluated. The study concluded that the technological applications of the surface system for medical devices and surgical tools, which contact human tissues for less than 1.5 h, are expected to be self-sterilizing without causing toxicity in vivo.

Sánchez-Salcedo, Sandra, et al. "**Design and preparation of biocompatible zwitterionic hydroxyapatite.**" *Journal of Materials Chemistry B* 1.11 (2013): 1595-1606.

This study reports the design and preparation of zwitterionic nanocrystalline hydroxyapatite (HA) capable of inhibiting bacterial adhesion while allowing osteoblast cell colonization. The surface functionalization of HA powders was carried out by post-synthesis grafting of 3-aminopropyltriethoxysilane (APTES) and carboxyethylsilanetriol sodium salt (CES) as amine and carboxylate precursors, respectively. The successful functionalization of HA surfaces was assessed by elemental chemical analysis, FTIR, ^{29}Si, ^{31}P and ^{13}C solid state CP/MAS NMR and ζ-potential measurements, and the zwitterionic nature of the synthesized HA was proved through the presence of $–NH_3^+/–COO^-$ pairs on the material surfaces. With the aim of evaluating the feasibility of this functionalization strategy in HA shaped in different physical forms, HA 3D macroporous scaffolds were fabricated by rapid prototyping and then provided with zwitterionic character. The effect of the simultaneous presence of $–NH_3^+/–COO^-$ zwitterionic pairs on the surface of HA on its behaviour regarding bacterial adhesion was tested using E. coli as model bacteria. The in vitro biocompatibility of these materials was investigated with cultured human HOS cells. The results indicate that it is possible to provide HA shaped in different physical forms (particles, granules, coatings, dense blocks, 3D scaffolds, etc.) with bacterial anti-adhesive properties via the “zwitterionization” process without affecting its biocompatibility. These findings open up promising expectations in many clinical fields

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including dentistry, maxillofacial surgery and otolaryngology, where a decrease in the bacterial adherence onto the implant surface would reduce the infection rates after implantation surgery.

Santis, R., et al. "**Advanced composites for hard-tissue engineering based on PCL/organic–inorganic hybrid fillers: From the design of 2D substrates to 3D rapid prototyped scaffolds.**" *Polymer Composites* 34.9 (2013): 1413-1417.

The bioactivity of sol–gel synthesized poly(ε-caprolactone) (PCL)/TiO2 or poly(ε-caprolactone)/ZrO2 particles was already known. In designing innovative 2D composite substrates for hard-tissue engineering, the possibility to embed PCL/TiO2 or PCL/ZrO2 hybrid fillers into a PCL matrix was previously proposed. In the present study, the potential of 3D fiber-deposition technique to design morphologically controlled scaffolds consisting of PCL reinforced with PCL/TiO2 or PCL/ZrO2 hybrid fillers was demonstrated. Finite element analysis was initially carried out on 2D substrates to find a correlation between the previously obtained results from the small punch test and the Young's modulus of the materials, whilst mechanical and biological tests were suitably performed on rapid prototyped scaffolds to assess the effects of the inclusion of the hybrid fillers on the performances of the 3D porous structures. The role of the inclusion of the hybrid fillers in improving the compressive modulus (about 90 MPa) and the cell viability/proliferation was demonstrated.

Shruti, Shruti, et al. "**Mesoporous bioactive scaffolds prepared with cerium-, gallium-and zinc-containing glasses.**" *Acta Biomaterialia* 9.1 (2013): 4836-4844.

Mesoporous bioactive glass scaffolds (MBG_Scs), based on 80% SiO2–15% CaO–5% P2O5 (in mol.%) mesoporous sol–gel glasses substituted with Ce2O3, Ga2O3 (both 0.2% or 1.0%) and ZnO (0.4% or 2.0%), were synthesized by combination of evaporation-induced self-assembly and rapid prototyping techniques. Cerium, gallium and zinc trace elements were selected because of their inherent beneficial biological properties. Fabricated scaffolds were characterized and compared with unsubstituted scaffold (B_Sc). All of them contained well interconnected ultralarge pores (pores >400 μm) ideal for vascular ingrowth and proliferation of cells. Macropores of size 100–400 μm were present inside the scaffolds. In addition, low-angle X-ray diffraction showed that B_Sc and scaffolds with substituent contents up to 0.4% exhibited ordered mesoporosity useful for hosting molecules with biological activity. The textural properties of B_Sc were a surface area of 398 m2 g−1, a pore diameter of 4.3 nm and a pore volume of 0.43 cm3 g−1. A slight decrease in surface area and pore volume was observed upon substitution with no distinct effect on pore diameter. In addition, all the MBG_Scs except 2.0% ZnO_Sc showed quite quick in vitro bioactive response. Hence, the present study is a positive addition to ongoing research into preparing bone tissue engineering scaffolds from bioceramics containing elements of therapeutic significance.

Wang, Min-Dan, et al. "**Novel crosslinked alginate/hyaluronic acid hydrogels for nerve tissue engineering.**" *Frontiers of Materials Science* 7.3 (2013): 269-284.

Artificial tissue engineering scaffolds can potentially provide support and guidance for the regrowth of severed axons following nerve injury. In this study, a hybrid biomaterial composed of alginate and hyaluronic acid (HA) was synthesized and characterized in terms of its suitability for covalent modification, biocompatibility for living Schwann cells and feasibility to construct three dimensional (3D) scaffolds. Carbodiimide mediated amide formation for the purpose of covalent crosslinking of the HA was carried out in the presence of calciumions that ionically crosslink alginate. Amide formation was found to be dependent on the concentrations of carbodiimide and calcium chloride. The double-crosslinked composite hydrogels display biocompatibility that is comparable to simple HA hydrogels, allowing for Schwann cell survival and growth. No significant difference was found between composite hydrogels made from different ratios of alginate and HA. A 3D BioPlotter™ rapid prototyping system was used to fabricate 3D scaffolds. The result indicated that combining HA with alginate facilitated the fabrication process and that 3D scaffolds with porous inner structure can be fabricated from the composite hydrogels, but not from HA alone. This information provides a basis for continuing in vitro

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and in vivo tests of the suitability of alginate/HA hydrogel as a biomaterial to create living cell scaffolds to support nerve regeneration.

Yilgor, P., et al. "**An in vivo study on the effect of scaffold geometry and growth factor release on the healing of bone defects.**" *Journal of tissue engineering and regenerative medicine* 7.9 (2013): 687-696.

The hypothesis of this study was that the extent of bone regeneration could be enhanced by using scaffolds with appropriate geometry, and that such an effect could be further increased by mimicking the natural timing of appearance of bone morphogenetic proteins BMP-2 and BMP-7 after fracture. Bioplotted poly(ε-caprolactone) (PCL) disks with four different fibre organizations were used to study the effect of 3D scaffold architecture on the healing of bone defects in a rat pelvis model. Moreover, one PCL construct was further modified by introducing a nanoparticulate sequential BMP-2/BMP-7 delivery system into this scaffold. Scaffolds and functionalized construct along with free nanocapsules were implanted using a rat iliac crest defect model. Six weeks post-implantation, the defects were evaluated by CT scan and histology. Analysis revealed that the basic architecture, having the highest pore volume for tissue ingrowth, presented the highest bone formation as determined by the bone mineral density (BMD) within the defect (144.2 ± 7.1); about four-fold higher than that of the empty defect (34.9 ± 10.7). It also showed the highest histological analysis scores with a high amount of bone formation within the defect, within the scaffold pores and along the outer surfaces of the scaffold. The basic scaffold carrying the BMP-2/BMP-7 delivery system showed significantly higher bone formation than the growth factor-free basic scaffold at 6 weeks (BMD 206.8 ± 15.7). Histological analysis also revealed new bone formation in close to or in direct contact with the construct interface. This study indicates the importance of open and interconnecting pore geometry on the better healing of bone defects, and that this effect could be further increased by supplying growth factors, as is the case in nature.

2014

Bakarich, Shannon E., et al. "**Printed ionic-covalent entanglement hydrogels from carrageenan and an epoxy amine.**" *RSC Advances* 4.72 (2014): 38088-38092.

Carrageenan/epoxy amine ionic-covalent entanglement hydrogels were fabricated on a 3D printer. The thermal gel transition behaviour of the biopolymer kappa-carrageenan was exploited to fix the shape of the patterned ink until a covalent polymer network formed by epoxy amine addition chemistry. The printed hydrogels display a work of extension value of 1.4 ± 0.3 MJ m^{-3}.

Bakarich, Shannon E. "**Three-Dimensional Printing Fiber Reinforced Hydrogel Composites.**" *ACS Appl. Mater. Interfaces* 6.18 (2014): 15998-16006.

An additive manufacturing process that combines digital modeling and 3D printing was used to prepare fiber reinforced hydrogels in a single-step process. The composite materials were fabricated by selectively pattering a combination of alginate/acrylamide gel precursor solution and an epoxy based UV-curable adhesive (Emax 904 Gel-SC) with an extrusion printer. UV irradiation was used to cure the two inks into a single composite material. Spatial control of fiber distribution within the digital models allowed for the fabrication of a series of materials with a spectrum of swelling behavior and mechanical properties with physical characteristics ranging from soft and wet to hard and dry. A comparison with the "rule of mixtures" was used to show that the swollen composite materials adhere to standard composite theory. A prototype meniscus cartilage was prepared to illustrate the potential application in bioengineering.

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Billiet, Thomas, et al. "**The 3D printing of gelatin methacrylamide cell-laden tissue-engineered constructs with high cell viability.**" *Biomaterials* 35.1 (2014): 49-62.

In the present study, we report on the combined efforts of material chemistry, engineering and biology as a systemic approach for the fabrication of high viability 3D printed macroporous gelatin methacrylamide constructs. First, we propose the use and optimization of VA-086 as a photo-initiator with enhanced biocompatibility compared to the conventional Irgacure 2959. Second, a parametric study on the printing of gelatins was performed in order to characterize and compare construct architectures. Hereby, the influence of the hydrogel building block concentration, the printing temperature, the printing pressure, the printing speed, and the cell density were analyzed in depth. As a result, scaffolds could be designed having a 100% interconnected pore network in the gelatin concentration range of 10–20 w/v%. In the last part, the fabrication of cell-laden scaffolds was studied, whereby the application for tissue engineering was tested by encapsulation of the hepatocarcinoma cell line (HepG2). Printing pressure and needle shape was revealed to impact the overall cell viability. Mechanically stable cell-laden gelatin methacrylamide scaffolds with high cell viability (>97%) could be printed.

Chang, Jae Won, et al. "**Tissue-Engineered Tracheal Reconstruction Using Three-Dimensionally Printed Artificial Tracheal Graft: Preliminary Report.**" *Artificial organs* 38.6 (2014): E95-E105.

Three-dimensional printing has come into the spotlight in the realm of tissue engineering. We intended to evaluate the plausibility of 3D-printed (3DP) scaffold coated with mesenchymal stem cells (MSCs) seeded in fibrin for the repair of partial tracheal defects. MSCs from rabbit bone marrow were expanded and cultured. A half-pipe-shaped 3DP polycaprolactone scaffold was coated with the MSCs seeded in fibrin. The half-pipe tracheal graft was implanted on a 10 × 10-mm artificial tracheal defect in four rabbits. Four and eight weeks after the operation, the reconstructed sites were evaluated bronchoscopically, radiologically, histologically, and functionally. None of the four rabbits showed any sign of respiratory distress. Endoscopic examination and computed tomography showed successful reconstruction of trachea without any collapse or blockage. The replaced tracheas were completely covered with regenerated respiratory mucosa. Histologic analysis showed that the implanted 3DP tracheal grafts were successfully integrated with the adjacent trachea without disruption or granulation tissue formation. Neo-cartilage formation inside the implanted graft was sufficient to maintain the patency of the reconstructed trachea. Scanning electron microscope examination confirmed the regeneration of the cilia, and beating frequency of regenerated cilia was not different from those of the normal adjacent mucosa. The shape and function of reconstructed trachea using 3DP scaffold coated with MSCs seeded in fibrin were restored successfully without any graft rejection.

Damanik, Febriyani FR, et al. "**Towards an in vitro model mimicking the foreign body response: tailoring the surface properties of biomaterials to modulate extracellular matrix.**" *Scientific reports* 4 (2014).

Despite various studies to minimize host reaction following a biomaterial implantation, an appealing strategy in regenerative medicine is to actively use such an immune response to trigger and control tissue regeneration. We have developed an in vitro model to modulate the host response by tuning biomaterials' surface properties through surface modifications techniques as a new strategy for tissue regeneration applications. Results showed tunable surface topography, roughness, wettability, and chemistry by varying treatment type and exposure, allowing for the first time to correlate the effect of these surface properties on cell attachment, morphology, strength and proliferation, as well as proinflammatory (IL-1β, IL-6) and antiflammatory cytokines (TGF-β1, IL-10) secreted in medium, and protein expression of collagen and elastin. Surface microstructuring, derived from chloroform partial etching, increased surface roughness and oxygen content. This resulted in enhanced cell adhesion, strength and proliferation as well as a balance of soluble factors for optimum collagen and elastin

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synthesis for tissue regeneration. By linking surface parameters to cell activity, we could determine the fate of the regenerated tissue to create successful soft tissue-engineered replacement.

Lee, Chang Hun, et al. "**3D Printed Multiphase Scaffolds for Regeneration of Periodontium Complex.**" *Tissue Engineering Part A* 20.7-8 (2014): 1342-1351.

Tooth-supporting periodontium forms a complex with multiple tissues, including cementum, periodontal ligament (PDL), and alveolar bone. In this study, we developed multiphase region-specific microscaffolds with spatiotemporal delivery of bioactive cues for integrated periodontium regeneration. Polycarprolactione-hydroxylapatite (90:10 wt%) scaffolds were fabricated using three-dimensional printing seamlessly in three phases: 100-μm microchannels in Phase A designed for cementum/dentin interface, 600-μm microchannels in Phase B designed for the PDL, and 300-μm microchannels in Phase C designed for alveolar bone. Recombinant human amelogenin, connective tissue growth factor, and bone morphogenetic protein-2 were spatially delivered and time-released in Phases A, B, and C, respectively. Upon 4-week in vitro incubation separately with dental pulp stem/progenitor cells (DPSCs), PDL stem/progenitor cells (PDLSCs), or alveolar bone stem/progenitor cells (ABSCs), distinctive tissue phenotypes were formed with collagen I-rich fibers especially by PDLSCs and mineralized tissues by DPSCs, PDLSCs, and ABSCs. DPSC-seeded multiphase scaffolds upon in vivo implantation yielded aligned PDL-like collagen fibers that inserted into bone sialoprotein-positive bone-like tissue and putative cementum matrix protein 1-positive/dentin sialophosphoprotein-positive dentin/cementum tissues. These findings illustrate a strategy for the regeneration of multiphase periodontal tissues by spatiotemporal delivery of multiple proteins. A single stem/progenitor cell population appears to differentiate into putative dentin/cementum, PDL, and alveolar bone complex by scaffold's biophysical properties and spatially released bioactive cues.

Lee, Chang H., et al. "**Protein-releasing polymeric scaffolds induce fibrochondrocytic differentiation of endogenous cells for knee meniscus regeneration in sheep.**" *Science Translational Medicine* 6.266 (2014): 266ra171-266ra171.

Regeneration of complex tissues, such as kidney, liver, and cartilage, continues to be a scientific and translational challenge. Survival of ex vivo cultured, transplanted cells in tissue grafts is among one of the key barriers. Meniscus is a complex tissue consisting of collagen fibers and proteoglycans with gradient phenotypes of fibrocartilage and functions to provide congruence of the knee joint, without which the patient is likely to develop arthritis. Endogenous stem/progenitor cells regenerated the knee meniscus upon spatially released human connective tissue growth factor (CTGF) and transforming growth factor–β3 (TGFβ3) from a three-dimensional (3D)–printed biomaterial, enabling functional knee recovery. Sequentially applied CTGF and TGFβ3 were necessary and sufficient to propel mesenchymal stem/progenitor cells, as a heterogeneous population or as single-cell progenies, into fibrochondrocytes that concurrently synthesized procollagens I and IIα. When released from microchannels of 3D-printed, human meniscus scaffolds, CTGF and TGFβ3 induced endogenous stem/progenitor cells to differentiate and synthesize zone-specific type I and II collagens. We then replaced sheep meniscus with anatomically correct, 3D-printed scaffolds that incorporated spatially delivered CTGF and TGFβ3. Endogenous cells regenerated the meniscus with zone-specific matrix phenotypes: primarily type I collagen in the outer zone, and type II collagen in the inner zone, reminiscent of the native meniscus. Spatiotemporally delivered CTGF and TGFβ3 also restored inhomogeneous mechanical properties in the regenerated sheep meniscus. Survival and directed differentiation of endogenous cells in a tissue defect may have implications in the regeneration of complex (heterogeneous) tissues and organs.

Naficy, Sina, et al. "**Graphene oxide dispersions: tuning rheology to enable fabrication.**" *Materials Horizons* 1.3 (2014): 326-331.

Here, we show that graphene oxide (GO) dispersions exhibit unique viscoelastic properties, making them a new class of soft materials. The fundamental insights accrued here provide the basis for the

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development of fabrication protocols for these two-dimensional soft materials, in a diverse array of processing techniques.

Nathan-Walleser, Teressa, et al. "**3D Micro-Extrusion of Graphene-based Active Electrodes: Towards High-Rate AC Line Filtering Performance Electrochemical Capacitors.**" *Advanced Functional Materials* 24.29 (2014): 4706-4716.

A facile one-step printing process by 3D micro-extrusion affording binder-free thermally reduced graphene oxide (TRGO) based electrochemical capacitors (ECs) that display high-rate performance is presented. Key intermediates are binder-free TRGO dispersion printing inks with concentrations up to 15 g L^{-1}. This versatile printing technique enables easy fabrication of EC electrodes, useful in both aqueous and non-aqueous electrolyte systems. The as-prepared TRGO material with high specific surface area (SSA) of 593 m2 g^{-1} and good electrical conductivity of ≈16 S cm^{-1} exhibits impressive charge storage performances. At 100 and 120 Hz, ECs fabricated with TRGO show time constants of 2.5 ms and 2.3 ms respectively. Very high capacitance values are derived at both frequencies ranging from 3.55 mF cm^{-2} to 1.76 mF cm^{-2}. Additionally, these TRGO electrodes can be charged and discharged at very high voltage scan rates up to 15 V s–1 yielding 4 F cm^{-3} with 50% capacitance retention. Electrochemical performance of TRGO electrodes in electrolyte containing tetraethyl ammonium tetrafluoroborate and acetonitrile (TEABF4-ACN) yields high energy density of 4.43 mWh cm^{-3} and power density up to 42.74 kW cm^{-3}, which is very promising for AC line filtering application and could potentially substitute state of the art electrolytic capacitor technology.

Neufurth, Meik, et al. "**Engineering a morphogenetically active hydrogel for bioprinting of bioartificial tissue derived from human osteoblast-like SaOS-2 cells.**" Biomaterials 35.31 (2014): 8810-8819.

Sodium alginate hydrogel, stabilized with gelatin, is a suitable, biologically inert matrix that can be used for encapsulating and 3D bioprinting of bone-related SaOS-2 cells. However, the cells, embedded in this matrix, remain in a non-proliferating state. Here we show that addition of an overlay onto the bioprinted alginate/gelatine/SaOS-2 cell scaffold, consisting of agarose and the calcium salt of polyphosphate [polyP·Ca^{2+}-complex], resulted in a marked increase in cell proliferation. In the presence of 100 µm polyP·Ca^{2+}-complex, the cells proliferate with a generation time of approximately 47–55 h. In addition, the hardness of the alginate/gelatin hydrogel substantially increases in the presence of the polymer. The reduced Young's modulus for the alginate/gelatin hydrogel is approximately 13–14 kPa, and this value drops to approximately 0.5 kPa after incubation of the cell containing scaffolds for 5 d. In the presence of 100 µm polyP·Ca^{2+}-complex, the reduced Young's modulus increases to about 22 kPa. The hardness of the polyP·Ca^{2+}-complex containing hydrogel remains essentially constant if cells are absent in the matrix, but it drops to 3.2 kPa after a 5 d incubation period in the presence of SaOS-2 cells, indicating that polyP·Ca^{2+}-complex becomes metabolized, degraded, by the cells. The alginate/gelatine-agarose system with polyP·Ca^{2+}-complex cause a significant increase in the mineralization of the cells. SEM analyses revealed that the morphology of the mineral nodules formed on the surface of the cells embedded in the alginate/gelatin hydrogel do not significantly differ from the nodules on cells growing in monolayer cultures. The newly developed technique, using cells encapsulated into an alginate/gelatin hydrogel and a secondary layer containing the morphogenetically active, growth promoting polymer polyP·Ca^{2+}-complex opens new possibilities for the application of 3D bioprinting in bone tissue engineering.

Rajaram, Ajay, David Schreyer, and Daniel Chen. "**Bioplotting Alginate/Hyaluronic Acid Hydrogel Scaffolds with Structural Integrity and Preserved Schwann Cell Viability.**" *3D Printing and Additive Manufacturing* 1.4 (2014): 194-203.

Bioplotting is an emerging freeform scaffold fabrication technique useful for creating artificial tissue scaffolds containing living cells. Simultaneous maintenance of scaffold structural integrity and cell

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viability is a challenging task. In this article, we present strategies developed to bioplot alginate-based three-dimensional tissue scaffolds containing hyaluronic acid and living Schwann cells for potential use in peripheral nerve tissue engineering. The fabrication platform, upon which the scaffold is created, was coated with the polycation polyethylenimine to immobilize the first layer of the scaffold on the platform. Each layer was then dispensed into a bath containing calcium chloride to cross-link the alginate, polyvinyl alcohol, and low concentrations of polyethylenimine to improve the structural integrity of the resulting scaffolds while retaining biocompatibility. The present study illustrated that with these strategies, porous alginate/hyaluronic acid scaffolds can be fabricated with good structural integrity and long-term cell viability. Plotting method and speed were also important factors determining the printability of single-layered scaffolds. The methods developed and the results obtained from this study form the basis for a general bioplotting method by which more sophisticated alginate/hyaluronic acid scaffolds can be fabricated with the inclusion of living cells into desired structures.

Rees, Adam, et al. "**3D bioprinting of carboxymethylated-periodate oxidized nanocellulose constructs for wound dressing applications.**" *BioMed Research International* (2014)

Nanocellulose has a variety of advantages, which make the material most suitable for use in biomedical devices such as wound dressings. The material is strong, allows for productionof transparent films, providesa moist wound healing environment and can form elastic gels with bio-responsive characteristics. In this study we explore the application of nanocellulose as a bioink for modifying film surfaces by a bioprinting process. Two different nanocelluloses were used, prepared with TEMPO mediated oxidation and a combination of carboxymethylation and periodate oxidation. The combination of carboxymethylation and periodate oxidation produced a homogeneous material with short nanofibrils, having widths < 20 nm and lengths < 200 nm. The small dimensions of the nanofibrils reduced the viscosity of the nanocellulose thus yielding a material with good rheological properties for use as a bioink. The nanocellulose bioink was thus used for printing 3D porous structures, which is exemplified in this study. We also demonstrated that both nanocelluloses inhibited bacterial growth, which is an interesting property of these novel materials.

Schumann, Paul, et al. "**Accelerating the early angiogenesis of tissue engineering constructs in vivo by the use of stem cells cultured in matrigel.**" *Journal of Biomedical Materials Research Part A* 102.6 (2014): 1652-1662.

In tissue engineering research, generating constructs with an adequate extent of clinical applications remains a major challenge. In this context, rapid blood vessel ingrowth in the transplanted tissue engineering constructs is the key factor for successful incorporation. To accelerate the microvascular development in engineered tissues, we preincubated osteoblast-like cells as well as mesenchymal stem cells or a combination of both cell types in Matrigel-filled PLGA scaffolds before transplantation into the dorsal skinfold chambers of balb/c mice. By the use of preincubated mesenchymal stem cells, a significantly accelerated angiogenesis was achieved. Compared with previous studies that showed a decisive increase of vascularization on day 6 after the implantation, we were able to halve this period and achieve explicitly denser microvascular networks 3 days after transplantation of the tissue engineering constructs. Thereby, the inflammatory host tissue response was acceptable and low, comparable with former investigations. A co- incubation of osteoblast-like cells and stem cells showed no additive effect on the density of the newly formed microvascular network. Preincubation of mesenchymal stem cells in Matrigel is a promising approach to develop rapid microvascular growth into tissue engineering constructs. After the implantation into the host organism, scaffolds comprising stem cells generate microvascular capillary-like structures exceptionally fast. Thereby, transplanted stem cells likely differentiate into vessel-associated cells. For this reason, preincubation of mesenchymal stem cells in nutrient solutions supporting different steps of angiogenesis provides a technique to promote the routine use of tissue engineering in the clinic.

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Wang, Xiaohong, Heinz C. Schröder, and Werner EG Müller. "**Biocalcite, a multifunctional inorganic polymer: Building block for calcareous sponge spicules and bioseed for the synthesis of calcium phosphate-based bone.**" *Beilstein Journal of Nanotechnology* 5.1 (2014): 610-621.

Calcium carbonate is the material that builds up the spicules of the calcareous sponges. Recent results revealed that the calcium carbonate/biocalcite-based spicular skeleton of these animals is formed through an enzymatic mechanism, such as the skeleton of the siliceous sponges, evolutionarily the oldest animals that consist of biosilica. The enzyme that mediates the calcium carbonate deposition has been identified as a carbonic anhydrase (CA) and has been cloned from the calcareous sponge species Sycon raphanus. Calcium carbonate deposits are also found in vertebrate bones besides the main constituent, calcium phosphate/hydroxyapatite (HA). Evidence has been presented that during the initial phase of HA synthesis poorly crystalline carbonated apatite is deposited. Recent data summarized here indicate that during early bone formation calcium carbonate deposits enzymatically formed by CA, act as potential bioseeds for the precipitation of calcium phosphate mineral onto bone-forming osteoblasts. Two different calcium carbonate phases have been found during CA-driven enzymatic calcium carbonate deposition in in vitro assays: calcite crystals and round-shaped vaterite deposits. The CA provides a new target of potential anabolic agents for treatment of bone diseases; a first CA activator stimulating the CA-driven calcium carbonate deposition has been identified. In addition, the CA-driven calcium carbonate crystal formation can be frozen at the vaterite state in the presence of silintaphin-2, an aspartic acid/glutamic acid-rich sponge-specific protein. The discovery that calcium carbonate crystals act as bioseeds in human bone formation may allow the development of novel biomimetic scaffolds for bone tissue engineering. Na-alginate hydrogels, enriched with biosilica, have recently been demonstrated as a suitable matrix to embed bone forming cells for rapid prototyping bioprinting/3D cell printing applications.

Wang, Xiaohong, et al. "**Effect of Bioglass on Growth and Biomineralization of SaOS-2 Cells in Hydrogel after 3D Cell Bioprinting.**" *PloS one* 9.11 (2014): e112497.

We investigated the effect of bioglass (bioactive glass) on growth and mineralization of bone-related SaOS-2 cells, encapsulated into a printable and biodegradable alginate/gelatine hydrogel. The hydrogel was supplemented either with polyphosphate (polyP), administered as polyP•Ca^{2+}-complex, or silica, or as biosilica that had been enzymatically prepared from ortho-silicate by silicatein. These hydrogels, together with SaOS-2 cells, were bioprinted to computer-designed scaffolds. The results revealed that bioglass (nano)particles, with a size of 55 nm and a molar ratio of SiO_2:CaO:P_2O_5 of 55:40:5, did not affect the growth of the encapsulated cells. If silica, biosilica, or polyP•Ca^{2+}-complex is co-added to the cell-containing alginate/gelatin hydrogel the growth behavior of the cells is not changed. Addition of 5 mg/ml of bioglass particles to this hydrogel significantly enhanced the potency of the entrapped SaOS-2 cells to mineralize. If compared with the extent of the cells to form mineral deposits in the absence of bioglass, the cells exposed to bioglass together with 100 µmoles/L polyP•Ca^{2+}-complex increased their mineralization activity from 2.1- to 3.9-fold, or with 50 µmoles/L silica from 1.8- to 2.9-fold, or with 50 µmoles/L biosilica from 2.7- to 4.8-fold or with the two components together (100 µmoles/L polyP•Ca^{2+}-complex and 50 µmoles/L biosilica) from 4.1- to 6.8-fold. Element analysis by EDX spectrometry of the mineral nodules formed by SaOS-2 revealed an accumulation of O, P, Ca and C, indicating that the mineral deposits contain, besides Ca-phosphate also Ca-carbonate. The results show that bioglass added to alginate/gelatin hydrogel increases the proliferation and mineralization of bioprinted SaOS-2 cells. We conclude that the development of cell-containing scaffolds consisting of a bioprintable, solid and cell-compatible inner matrix surrounded by a printable hard and flexible outer matrix containing bioglass, provide a suitable strategy for the fabrication of morphogenetically active and biodegradable implants.

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Zhang, Jianhua, et al. "**3D-Printed Magnetic Fe3O4/MBG/PCL Composite Scaffolds with Multifunctionality of Bone Regeneration, Local Anticancer Drug Delivery and Hyperthermia.**" *Journal of Materials Chemistry B* 2.43 (2014): 7583-7595.

In this study, three-dimensional (3D) magnetic Fe3O4 nanoparticles containing mesoporous bioactive glass/polycaprolactone (Fe3O4/MBG/PCL) composite scaffolds have been fabricated by 3D-printing technique. The physiochemical properties, in vitro bioactivity, anticancer drug delivery, mechanical strength, magnetic heating ability and cell response to Fe3O4/MBG/PCL scaffolds were systematically investigated. The results showed that Fe3O4/MBG/PCL scaffolds had uniform macropores of 400 μm, high porosity of 60 % and excellent compressive strength of 13-16 MPa. The incorporation of magnetic Fe3O4 nanoparticles into MBG/PCL scaffolds did not influence their apatite mineralization ability, but endowed excellent magnetic heating ability and significantly stimulated proliferation, alkaline phosphatase (ALP) activity, osteogenesis-related gene expression (RUNX2, OCN, BSP, BMP-2 and Col-1) and extra-cellular matrix (ECM) mineralization of human bone marrow-derived mesenchymal stem cells (h-BMSCs). Moreover, using doxorubicin (DOX) as a model anticancer drug, Fe3O4/MBG/PCL scaffolds exhibited a sustained drug release for use in local drug delivery therapy. Therefore, the 3D-printed Fe3O4/MBG/PCL scaffolds showed the potential multifunctionality of enhanced osteogenic activity, local anticancer drug delivery and magnetic hyperthermia.

Zhang, Jianhua, et al. "**Three-dimensional printing of strontium-containing mesoporous bioactive glass scaffolds for bone regeneration.**" *Acta biomaterialia* 10.5 (2014): 2269-2281.

In this study, we fabricated strontium-containing mesoporous bioactive glass (Sr-MBG) scaffolds with controlled architecture and enhanced mechanical strength using a three-dimensional (3-D) printing technique. The study showed that Sr-MBG scaffolds had uniform interconnected macropores and high porosity, and their compressive strength was ~170 times that of polyurethane foam templated MBG scaffolds. The physicochemical and biological properties of Sr-MBG scaffolds were evaluated by ion dissolution, apatite-forming ability and proliferation, alkaline phosphatase activity, osteogenic expression and extracelluar matrix mineralization of osteoblast-like cells MC3T3-E1. The results showed that Sr-MBG scaffolds exhibited a slower ion dissolution rate and more significant potential to stabilize the pH environment with increasing Sr substitution. Importantly, Sr-MBG scaffolds possessed good apatite-forming ability, and stimulated osteoblast cells' proliferation and differentiation. Using dexamethasone as a model drug, Sr-MBG scaffolds also showed a sustained drug delivery property for use in local drug delivery therapy, due to their mesoporous structure. Therefore, the 3-D printed Sr-MBG scaffolds combined the advantages of Sr-MBG such as good bone-forming bioactivity, controlled ion release and drug delivery and enhanced mechanical strength, and had potential application in bone regeneration.

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Akbarzadeh, Rosa, et al. "**Hierarchical polymeric scaffolds support the growth of MC3T3-E1 cells.**" *Journal of Materials Science: Materials in Medicine* 26.2 (2015): 1-12.

Tissue engineering makes use of the principles of biology and engineering to sustain 3D cell growth and promote tissue repair and/or regeneration. In this study, macro/microporous scaffold architectures have been developed using a hybrid solid freeform fabrication/thermally induced phase separation (TIPS) technique. Poly(lactic-co-glycolic acid) (PLGA) dissolved in 1,4-dioxane was used to generate a microporous matrix by the TIPS method. The 3D-bioplotting technique was used to fabricate 3D macroporous constructs made of polyethylene glycol (PEG). Embedding the PEG constructs inside the PLGA solution prior to the TIPS process and subsequent extraction of PEG following solvent removal (1,4-dioaxane) resulted in a macro/microporous structure. These hierarchical scaffolds with a bimodal pore size distribution (<50 and >300 µm) contained orthogonally interconnected macro-channels generated by the extracted PEG. The diameter of the macro-channels was varied by tuning the dispensing parameters of the 3D bioplotter. The in vitro cell culture using murine MC3T3-E1 cell line for 21 days demonstrated that these scaffolds could provide a favorable environment to support cell adhesion and growth.

Bakarich, Shannon E., Robert Gorkin, and Geoffrey M. Spinks. "**4D Printing with Mechanically Robust, Thermally Actuating Hydrogels.**" *Macromolecular Rapid Communications* (2015).

A smart valve is created by 4D printing of hydrogels that are both mechanically robust and thermally actuating. The printed hydrogels are made up of an interpenetrating network of alginate and poly(N-isopropylacrylamide). 4D structures are created by printing the “dynamic” hydrogel ink alongside other static materials.

Bawolin, Nahshon K., et al. "Characterization Of Mechanical Properties Of Tissue Scaffolds By Phase Contrast Imaging And Finite Element Modeling." (2015).

In tissue engineering, the cell and scaffold approach has shown promise as a treatment to regenerate diseased and/or damaged tissue. In this treatment, an artificial construct or scaffold is seeded with compatible cells, which then proceed to organize and proliferate into new tissue. The scaffold itself then biodegrades with time, eventually leaving behind only the newly-formed tissue. The degradation qualities of the scaffold are critical to its performance during the treatment period, since the change in the mechanical properties of the scaffold with its degradation can have a significant influence on cell behavior. To observe in time the scaffold's mechanical properties, a straightforward method is to deform the scaffold and then characterize the deflection of the scaffold accordingly. However, experimentally observing the deflection the scaffold experiences has shown as a challenging task. To overcome this challenge, this paper presents a novel study on the characterization of mechanical properties of tissue scaffolds by radiation force impulse and phase contract imaging and their finite element modeling, which specifically includes scaffold fabrication, scaffold imaging, image analysis, and finite elements modeling of the scaffold mechanical properties. The innovation of the work rests on the use of in-line phase contrast x-ray imaging at 20 KeV to characterize tissue scaffold deformation caused by ultrasound radiation forces, and the use of the Fourier transform to identify sub pixel movement. Once deformation has been determined experimentally, it is then compared with the prediction given by the forward solution of a finite element model. A consideration of the number of separate loading conditions necessary to uniquely identify the material properties of transversely isotropic and fully orthotropic scaffolds is also presented.

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Higuera, Gustavo A., et al. "**Spatiotemporal proliferation of human stromal cells adjusts to nutrient availability and leads to stanniocalcin-1 expression in vitro and in vivo.**" *Biomaterials* 61 (2015): 190-202.

Cells and tissues are intrinsically adapted to molecular gradients and use them to maintain or change their activity. The effect of such gradients is particularly important for cell populations that have an intrinsic capacity to differentiate into multiple cell lineages, such as bone marrow derived mesenchymal stromal cells (MSCs). Our results showed that nutrient gradients prompt the spatiotemporal organization of MSCs in 3D culture. Cells adapted to their 3D environment without significant cell death or cell differentiation. Kinetics data and whole-genome gene expression analysis suggest that a low proliferation activity phenotype predominates in stromal cells cultured in 3D, likely due to increasing nutrient limitation. These differences implied that despite similar surface areas available for cell attachment, higher cell concentrations in 3D reduced MSCs proliferation, while activating hypoxia related-pathways. To further understand the in vivo effects of both proliferation and cell concentrations, we increased cell concentrations in small (1.8 ml) implantable wells. We found that MSCs accumulation and conditioning by nutrient competition in small volumes leads to an ideal threshold of cell-concentration for the induction of blood vessel formation, possibly signaled by the hypoxia-related stanniocalcin-1 gene.

Jakus, Adam E., et al. "**Three Dimensional Printing of High-Content Graphene Scaffolds for Electronic and Biomedical Applications.**" *ACS Nano* (2015).

The exceptional properties of graphene enable applications in electronics, optoelectronics, energy storage, and structural composites. Here we demonstrate a 3D printable graphene (3DG) composite consisting of majority graphene and minority polylactide-co-glycolide, a biocompatible elastomer, 3D printed from a liquid ink. This ink can be utilized under ambient conditions via extrusion-based 3D printing to create graphene structures with features as small as 100 μm comprised of as few as two layers (<300 μm thick object) or many hundreds of layers (>10 cm thick object). The resulting 3DG material is mechanically robust and flexible while retaining electrical conductivities greater than 800 S/m, an order of magnitude increase over previously reported 3D printed carbon materials. In vitro experiments in simple growth medium, in the absence of neurogenic stimuli, reveal that 3DG supports human mesenchymal stem cell (hMSC) adhesion, viability, proliferation, and neurogenic differentiation with significant upregulation of glial and neuronal genes. This coincides with hMSCs adopting highly elongated morphologies with features similar to axons and presynaptic terminals. In vivo experiments indicate that 3DG has promising biocompatibility over the course of at least 30 days. Surgical tests using a human cadaver nerve model also illustrate that 3DG has exceptional handling characteristics and can be intraoperatively manipulated and applied to fine surgical procedures. With this unique set of properties, combined with ease of fabrication, 3DG could be applied towards the design and fabrication of a wide range of functional electronic, biological, and bioelectronic medical and non-medical devices.

Kim, Yoo Suk, et al. "**The Application of Three-Dimensional Printing in Animal Model of Augmentation Rhinoplasty.**" *Annals of Biomedical Engineering* (2015): 1-10.

The role of three-dimensional (3D) printing has expanded in diverse areas in medicine. As plastic surgery needs to fulfill the different demands from diverse individuals, the applications of tailored 3D printing will become indispensable. In this study, we evaluated the feasibility of using 3D-printed polycaprolactone (PCL) scaffold seeded with fibrin/chondrocytes as a new dorsal augmentation material for rhinoplasty. The construct was surgically implanted on the nasal dorsum in the subperiosteal plane of six rabbits. The implants were harvested 4 and 12 weeks after implantation and evaluated by gross morphological assessment, radiographic imaging, and histologic examination. The initial shape of the implant was unchanged in all cases, and no definite post-operative complications were seen over the 3-month period. Radiologic evaluation confirmed that implants remained in the initial location without migration or extrusion. Histologic evaluations showed that the scaffold architectures were maintained with minimal inflammatory reactions; however, expected neo-chondrogenesis was not definite in the

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constructs. A new PCL scaffold designed by 3D printing method seeded with fibrin/chondrocytes can be a biocompatible augmentation material in rhinoplasty in the future.

Li, Kun, et al. "**Three-dimensionally plotted MBG/PHBHHx composite scaffold for antitubercular drug delivery and tissue regeneration.**" *Journal of Materials Science: Materials in Medicine* 26.2 (2015): 1-8.

A suitable drug-loaded scaffold that can postoperatively release an antituberculosis drug efficiently in a lesion area and help repair a bone defect is very important in the clinical treatment of bone tuberculosis (TB). In this study, a composite drug-loaded cylindrical scaffold was prepared by using three-dimensional printing technology in combination with the mesoporous confinement range, surface chemical groups, and gradual degradation of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). This achieves the slow release of a drug for as long as possible. We implanted the drug-loaded compound scaffold into New Zealand rabbits' femur defect model to study the in vivo drug release performance and osteogenic ability. The in vivo release of isoniazid and rifampicin from the prepared composites could be effectively sustained for 12 weeks in local tissues, whereas these drugs were sustained for just 2 weeks in a control group. The blood drug concentrations were very low and most concentrations were below 5 μg/ml. Therefore, the systemic toxic adverse effect is very low. In addition, the composite exhibits good osteogenic potential in a rabbit bone defect model. The results of this study indicate that this composite has great potential for treating osteoarticular TB.

Martínez-Vázquez, F. J., et al. "**Fabrication of novel Si-doped Hydroxyapatite/Gelatine scaffolds by rapid prototyping for drug delivery and bone regeneration.**" *Acta Biomaterialia* (2015).

Porous 3-D scaffolds consisting of gelatine and Si-doped hydroxyapatite were fabricated at room temperature by rapid prototyping. Microscopic characterization revealed a highly homogeneous structure, showing the pre-designed porosity (macroporosity) and a lesser in-rod porosity (microporosity). The mechanical properties of such scaffolds are close to those of trabecular bone of the same density. The biological behavior of these hybrid scaffolds is greater than that of pure ceramic scaffolds without gelatine, increasing pre-osteoblastic MC3T3-E1 cell differentiation (matrix mineralization and gene expression).Since the fabrication process of these structures was carried out at mild conditions, an antibiotic (vancomycin) was incorporated in the slurry before the extrusion of the structures. The release profile of this antibiotic was measured in phosphate buffered saline solution by High-Performance Liquid Chromatography and was adjusted to a first order release kinetics. Released vancomycin from the material was also shown to inhibit bacterial growth in vitro. The implications of the result showed in this study for bone tissue engineering applications are discussed.

Min, Zhu, et al. "**3D-printed hierarchical scaffold for localized isoniazid/rifampin drug delivery and osteoarticular tuberculosis therapy.**" *Acta Biomaterialia* (2015).

After surgical treatment of osteoarticular tuberculosis (TB), it is necessary to fill the surgical defect with an implant, which combines the merits of osseous regeneration and local multi-drug therapy so as to avoid drug resistance and side effects. In this study, a 3D-printed macro/meso-porous composite scaffold is fabricated. High dosages of isoniazid (INH)/rifampin (RFP) anti-TB drugs are loaded into chemically modified mesoporous bioactive ceramics in advance, which are then bound with poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) through a 3D printing procedure. The composite scaffolds show greatly prolonged drug release time compared to commercial calcium phosphate scaffolds either in vitro or in vivo. In addition, the drug concentrations on the periphery tissues of defect are maintained above INH/RFP minimal inhibitory concentrations even up to 12 weeks post-surgery, while they are extremely low in blood. Examinations of certain serum enzymes suggest no harm to hepatic or renal functions. Micro-CT evaluations and histology results also indicate partly degradation of the composite scaffolds and new bone growth in the cavity. These results suggest promising applications

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of our hierarchical composite scaffold in bone regeneration and local anti-TB therapy after osteoarticular TB debridement surgery.

Mota, Carlos, et al. "**Multiscale fabrication of biomimetic scaffolds for tympanic membrane tissue engineering.**" *Biofabrication* 7.2 (2015): 025005.

The tympanic membrane (TM) is a thin tissue able to efficiently collect and transmit sound vibrations across the middle ear thanks to the particular orientation of its collagen fibers, radiate on one side and circular on the opposite side. Through the combination of advanced scaffolds and autologous cells, tissue engineering (TE) could offer valuable alternatives to autografting in major TM lesions. In this study, a multiscale approach based on electrospinning (ES) and additive manufacturing (AM) was investigated to fabricate scaffolds, based on FDA approved copolymers, resembling the anatomic features and collagen fiber arrangement of the human TM. A single scale TM scaffold was manufactured using a custom-made collector designed to confer a radial macro-arrangement to poly(lactic-co-glycolic acid) electrospun fibers during their deposition. Dual and triple scale scaffolds were fabricated combining conventional ES with AM to produce poly(ethylene oxide terephthalate)/poly(butylene terephthalate) block copolymer scaffolds with anatomic-like architecture. The processing parameters were optimized for each manufacturing method and copolymer. TM scaffolds were cultured in vitro with human mesenchymal stromal cells, which were viable, metabolically active and organized following the anisotropic character of the scaffolds. The highest viability, cell density and protein content were detected in dual and triple scale scaffolds. Our findings showed that these biomimetic micro-patterned substrates enabled cell disposal along architectural directions, thus appearing as promising substrates for developing functional TM replacements via TE.

Müller, W. E. G., et al. "**A new printable and durable N,O-carboxymethyl chitosan-Ca2+-polyphosphate complex with morphogenetic activity**" *Journal of Materials Chemistry B* (2015).

Biomimetic materials gain increasing importance in tissue engineering since they may provide regenerative alternatives to the use of autologous tissues for transplantation. In the present study we applied for bioprinting of a functionalized three-dimensional template, N,O-carboxymethyl chitosan (N,O-CMC), mimicking the physiological extracellular matrix. This polymer, widely used in tissue engineering, has been provided with functional activity by integration of polyphosphate (polyP), an osteogenically acting natural polymer. The two polymers, N,O-CMC and polyP, are linked together via Ca2+ bridges. This N,O-CMC+polyP material proved to be printable and durable. The N,O-CMC+polyP printed layers and tissue units retain their property to induce SaOS-2 bone-like cells to biomineralization. Subsequent in vivo experiments revealed a strong regeneration-inducing activity of the material in the rat calvarial defect model. In turn, N,O-CMC+polyP represents a promising hybrid material useful as potential custom-designed scaffold for alternative tissue-engineering solutions.

Murray, E., et al. "**A bio-friendly, green route to processable, biocompatible graphene/polymer composites.**" *RSC Advances* 5.56 (2015): 45284-45290.

Graphene-based polymer composites are a very promising class of compounds for tissue engineering scaffolds. However, in general the methods of synthesis are environmentally hazardous and residual toxic materials can affect the biocompatibility significantly. In this paper a simple, scalable, environmentally-friendly, microwave-assisted synthesis is described that results in conducting graphene/polycaprolactone composites that retain the processability and biocompatibility of the pristine polymer without introducing possibly hazardous reducing agents. Composites of polycaprolactone and graphene oxide were synthesised in a single step by the ring-opening polymerisation of ε-caprolactone in the presence of dispersed graphene oxide nanosheets under microwave irradiation. The graphene oxide provides a nucleation centre for the crystallisation of the polymer resulting in polymer-functionalised nanosheets. During polymerisation, the graphene oxide was also reduced to conducting graphene. The resulting graphene/polymer composites were comparable to composites prepared by blending previously highly chemically reduced graphene into polycaprolactone,

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and they could be easily dispersed in a number of solvents or melt extruded for further processing. These three-dimensional melt extruded materials showed excellent biocompatibility and are promising substrates for tissue engineering scaffolds.

Rajaram, Ajay, David J. Schreyer, and Daniel XB Chen. "**Use of the polycation polyethyleneimine to improve the physical properties of alginate-hyaluronic acid hydrogel during fabrication of tissue repair scaffolds.**" *Journal of Biomaterials Science, Polymer Edition* just-accepted (2015): 1-23.

Recently alginate-based tissue repair scaffolds fabricated using 3D printing techniques have been extensively examined for use in tissue engineering applications. However, their physical and mechanical properties are unfavorable for many tissue engineering applications because these properties are poorly controlled during the fabrication process. Some improvement of alginate gel properties can be realized by addition of hyaluronic acid (HA), and this may also improve the ability of cells to interact with the gel. Here we report improvement of the physical properties of alginate-HA gel scaffolds by the addition of the polycation polyethyleneimine (PEI) during the fabrication process in order to stabilize alginate molecular structure through the formation of a polyelectrolyte complex. We find that PEI has a significant beneficial influence on alginate-HA scaffold physical properties, including a reduction in the degree of gel swelling, a reduction in scaffold degradation rate, and an increase in the Young's modulus of the gel. Further study shows that fabrication of alginate-HA gels with PEI increases the encapsulation efficiency of bovine serum albumin, a model protein, and reduces the subsequent initial protein release rate. However, it was also found that survival of Schwann cells or ATDC-5 chondrogenic cells encapsulated during the scaffold fabrication process was modestly reduced with increasing PEI concentration. This study illustrates that the use of PEI during scaffold fabrication by plotting can provide an effective means to control alginate-based scaffold properties for tissue engineering applications, but that the many effects of PEI must be balanced for optimal outcomes in different situations.

Rutz, Alexandra L., et al. "**A Multimaterial Bioink Method for 3D Printing Tunable, Cell-Compatible Hydrogels.**" *Advanced Materials* (2015).

A multimaterial bioink method using polyethylene glycol crosslinking is presented for expanding the biomaterial palette required for 3D bioprinting more mimetic and customizable tissue and organ constructs. Lightly crosslinked, soft hydrogels are produced from precursor solutions of various materials and 3D printed. Rheological and biological characterizations are presented, and the promise of this new bioink synthesis strategy is discussed.

Salinas, Antonio J., and María Vallet-Regí. "**Glasses in bone regeneration: A multiscale issue.**" *Journal of Non-Crystalline Solids* (2015).

3D scaffolds based in mesoporous bioactive glasses (MBGs) are being widely investigated to use in bone tissue engineering (TE) applications. These scaffolds are often obtained by rapid prototyping (RP) and exhibit an array of interconnected pores in a hierarchy of sizes. The ordered mesopore network (around 4 nm in diameter) is optimal for the adsorption and release of bone inductor biomolecules, and the arrangement of macropores over 100 μm facilitates the bone cell ingrowths and angiogenesis. Nevertheless MBG composition can be varied almost infinitely at the atomic scale by including in the glass network oxides of inorganic elements with a therapeutic action. In this article the synthesis and characterization of MBG scaffolds based on the 80%SiO_2–15%CaO–5%P_2O_5 (in mol-%) glass with substitutions up to 3.5% of Ga_2O_3 or Ce_2O_3 or 7.0% of ZnO are revisited. The substituent inclusion and the RP processing slightly decrease the surface area, the pore volume and the mesoporous order as well as their bioactive response in solutions mimicking blood plasma. However, these values still remain useful for bone TE applications. Results exhibiting the bactericide action of MBG scaffolds containing ZnO are also presented.

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Sheshadri, Priyanka, and Rohan A. Shirwaiker. "**Characterization of Material–Process–Structure Interactions in the 3D Bioplotting of Polycaprolactone.**" *3D Printing and Additive Manufacturing* 2.1 (2015): 20-31.

Three-dimensional (3D) bioplotting is a melt-extrusion-based additive manufacturing process used to fabricate 3D scaffolds for tissue engineering applications. This study investigates the relationship between material rheology, process parameters, and scaffold characteristics during 3D bioplotting of polycaprolactone (PCL). The effects of two process parameters, extrusion temperature and nozzle diameter, on resultant scaffold structure and compression strength were studied using design of experiments. PCL scaffolds designed for a 24-well culture plate (Ø 14 mm × 2 mm) were bioplotted in a 0°/90° laydown pattern at three levels of extrusion temperature (80°C, 90°C, and 100°C) and two levels of nozzle inner diameter (0.3 and 0.4 mm) at a constant extrusion pressure (0.35 N/mm^2) and nozzle speed (1.2 mm/s). The relationship between PCL dynamic viscosity and extrusion temperature during bioplotting was then determined using rheological measurements. The ANOVA results demonstrated that the strand width and porosity significantly varied as a function of the extrusion temperature, the nozzle inner diameter, and the interaction of the two parameters ($p < 0.05$). The compression modulus of scaffolds fabricated at the different experimental levels also showed an increasing trend (0.5 – 0.97 MPa) with extrusion temperature for a given nozzle diameter. Rheological analysis at the three extrusion temperatures showed the average viscosity to be significantly different at each level ($p < 0.05$). The apparent viscosity of PCL at the nozzle tip during 3D bioplotting was also estimated, showing a steep decrease with increasing extrusion temperature and nozzle diameter. Finally, predictive regression models to estimate the scaffold characteristics based on the 3D bioplotting process parameters were developed.

Schirmer, Katharina SU, et al. "**From nanoparticles to fibres: effect of dispersion composition on fibre properties.**" *Journal of Nanoparticle Research* 17.6 (2015): 1-11.

A polyvinyl alcohol (PVA)-stabilized polypyrrole nanodispersion has been optimised for conductivity and processability by decreasing the quantity of PVA before and after synthesis. A reduction of PVA before synthesis leads to the formation of particles with a slight increase in dry particle diameter (51 ± 6 to 63 ± 3 nm), and conversely a reduced hydrodynamic diameter. Conductivity of the dried nanoparticle films was not measureable after a reduction of PVA prior to synthesis. Using filtration of particles after synthesis, PVA content was sufficiently reduced to achieve dried thin film conductivity of 2 S cm^{-1}, while the electroactivity of the dispersed particles remained unchanged. The as-synthesized and PVA-reduced polypyrrole particles were successfully spun into all-nanoparticle fibres using a wet-extrusion approach without the addition of any polymer or gel matrix. Using nanoparticles as a starting material is a novel approach, which allowed the production of macro-scale fibres that consisted entirely of polypyrrole nanoparticles. Fibres made from PVA-reduced polypyrrole showed higher electroactivity compared to fibres composed of the dispersion high in PVA. The mechanical properties of the fibres were also improved by reducing the amount of PVA present, resulting in a stronger, more ductile and less brittle fibre, which could find potential application in various fields.

Yuan, Jing, et al. "**The preliminary performance study of the 3D printing of a tricalcium phosphate scaffold for the loading of sustained release anti-tuberculosis drugs**." *Journal of Materials Science* 50.5 (2015): 2138-2147.

In the surgical treatment of tuberculosis of the bones, excision of the lesion site leaves defects in the bone structure. Recent research has shown benefits for bone tissue support, such as tricalcium phosphate, as regrowth materials. These biocompatible engineering materials have good bone inductivity and biologic mechanical performance. The goal of this study was to evaluate the use of 3D printing, a new technology, to design and build 3-dimensional support structures for use in grafting at lesion sites and for use in embedding the sustained release anti-tuberculosis drugs Rifampin and

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Isoniazid and determine the in vivo performance of these structures. In addition to mechanical studies, osteogenesis, cell viability, and migration were all observed, using Wistar rat models, to determine the effectiveness of this material as a biological support. The bone support showed good resistance to compression, similar to the spongiest bone tissue, and high porosity. In vivo studies showed that the material had a stable time release of Rifampin and Isoniazid through 90 days and achieved effective killing of the tuberculosis-causing bacteria. Finally, the support allowed for good migration and survival of rat bone marrow mesenchymal stem cells, leading to successful bone regrowth and repair. These results imply that the use of 3D printing of tricalcium phosphate scaffolds for bone excision repair and time-release treatment of tuberculosis shows great promise for future treatment of patients with tuberculosis of the bones.

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Zhao, Shichang, et al. "**Three-dimensional printed strontium-containing mesoporous bioactive glass scaffolds for repairing rat critical-sized calvarial defects.**" *Acta biomaterialia* 12 (2015): 270-280.

The development of a new generation of biomaterials with high osteogenic ability for fast osseointegration with host bone is being intensively investigated. In this study, we have fabricated three-dimensional (3-D) strontium-containing mesoporous bioactive glass (Sr-MBG) scaffolds by a 3-D printing technique. Sr-MBG scaffolds showed uniform interconnected macropores (~400 μm), high porosity (~70%) and enhanced compressive strength (8.67 ± 1.74 MPa). Using MBG scaffolds as a control, the biological properties of Sr-MBG scaffolds were evaluated by apatite-forming ability, adhesion, proliferation, alkaline phosphatase activity and osteogenic gene expression of osteoblast-like cells MC3T3-E1. Furthermore, Sr-MBG scaffolds were used to repair critical-sized rat calvarial defects. The results showed that Sr-MBG scaffolds possessed good apatite-forming ability and stimulated MC3T3-E1 cell proliferation and differentiation. Importantly, the in vivo results revealed that Sr-MBG scaffolds had good osteogenic capability and stimulated new blood vessel formation in critical-sized rat calvarial defects within 8 weeks. Therefore, 3-D printed Sr-MBG scaffolds with favorable pore structure and high osteogenic ability have more potential applications in bone regeneration.

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