exhibiting 3D patterns of multiple kinds of functional molecules. The strategy is simple, hydrogel-independent, versatile, and does not require sophisticated equipment or chemical reactions. The technique permits the fabrication of precise, multifunctional and well-defined patterns of different molecules (such as peptides and proteins) with micron scale resolution and up to centimetres in depth.

Results: Cell culture studies revealed selective molecular recognition inducing cell penetration into hydrogels, demonstrating that the patterned molecules are functional after the fabrication process.

Discussion: The capacity to manipulate and localize multiple proteins in their native state replicating geometrical configurations found in biological systems would benefit applications that require fine control of molecular and cell organisation. We believe that the device has the potential to grow into a novel material platform technology, enabling generation of hybrid multifunctional environments which can serve as a new standard in biomimetic cell culture studies.

Bioprinting Technology as a Tool for Building Complex Tissues and Organs

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Advances in tissue engineering and regenerative medicine have led to the development of many clinical therapies. However, challenges still exist in developing complex tissue systems. One challenge that hampers rapid clinical translation is the lack of effective delivery methods for cells and biomaterials to build complex tissue constructs. Living tissues maintain inherent multi-cellular heterogeneous structures, and rebuilding of such complex tissue structures requires subtle arrangements of different cell types and extracellular matrices at their specific anatomical target sites. 3D bioprinting has emerged as an innovative technology that has the potential to address this endeavor. In this session novel and versatile approaches to building tissue structures using 3D printing technology will be discussed. Clinical perspectives unique to 3D printed structures will also be discussed.

Biofabrication Strategies in Otosurgery: From the Outer to the Inner Ear

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Ear physiology occurs via minute highly-specialized and histodiverse tissue components with a shape-dependent function, which ultimately allow a fine hearing. Such an anatomic site is thus a challenging application for tissue-engineers in which microfabrication techniques can make a difference. The aim of this study was to develop specific biofabrication strategies to replace the ear tissues, which could be useful in otologic surgery. 3D fiber deposition (3DF) was used as an additive manufacturing technique to obtain ear bone replacements, such as outer auditory canal wall and ossicular chain (OC), based on poly(ethylene oxide terephthalate)/poly(butylene terephthalate) (PEOT/PBT) copolymer. These scaffolds were cultured with human mesenchymal stromal cells entrapped in fibrin clots as a biological nanofibrous matrix. After 21–27 days of osteodifferentiation, cell viability, bone markers and microCT were performed to assess appropriate mineralization. The measured acoustic response of OC constructs was superior to those of commercial prostheses in the hearing ranges: 250–8.000 Hz frequencies and 50–100 dB sound pressures. The tympanic membrane is a flexible and though connective tissue apt for vibration. Electrospinning was used in combination with 3DF to produce biomimetic PEOT/PBT dual and triple scale scaffolds provided with over-impressed patterning (radial, circular and reticular) able to localize cells and their synthesized biomolecules as in the native eardrum. The possibility of producing electrospun meshes that enable cell alignment was also investigated via a radial collector. Finally, other biofabrication strategies were investigated to produce thin ceramic/polymer composite scaffolds able to support the inner ear function, including spin coating, hot-press, and co-axial electrospinning.

3D Printed Nanocellulose Threads for Delivering Human Stem Cell inside Wounds

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One of the main issues in regenerative therapies is still the lack of appropriate vehicles for delivering cells into wounded areas effectively and safely. Additionally, cell application in surgery has traditionally relied on animal-origin constituents that may induce immune reactions or infections. To solve these problems, we propose 3D printed and cross-linked sutures of wood-derived nanofibrillar cellulose (NFC) to deliver immunomodulatory xenogeneic-free cells inside wounds.

GrowDex NFC, received as hydrogel, was printed into an ethanol bath by using a Fab@home 3D printer. After that, threads were cross-linked with a solution consisting in glutaraldehyde and zinc nitrate. The threads were sterilized before human adipose-derived stem cells (hASCs) (n = 4) were seeded and cultured. Viability, toxicity of threads over the hASCs cells was analysed. Molecular and cellular *in-vitro* studies were performed in order to detect changes in the hASCs profile or bioactivity. Suture strength was also tested through skin layers in both *ex-vivo* and *in-vivo* pig model.

Our study showed cross-linked NFC threads presented high mechanical strength even under the wet surgery conditions. Moreover hASCs attached over the 3D printed threads without showing any evidence of toxicity or proliferation reduction. Cell morphology and undifferentiated state were also maintained as well as their bioactivity concerning cytokine's pattern.

As a proof of concept, we demonstrate herein the possibility to create functionalized surgical sutures using 3D printed cross-linked NFC threads decorated with hASCs. Our findings highlight the use of these bio-sutures as a promising tool for fighting against post-chirurgical inflammation and chronic wound situations.

Non-viral Gene Delivery within 3D Bioprinted PCL-Reinforced Alginate Hydrogels for Bone Regeneration

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The combination of gene delivery technology with 3D-Bioprinting offers a promising platform for musculoskeletal tissue engineering. The overarching goal of this approach is to generate an ''off-the-shelf'', mechanically functional gene delivery system for bone repair. Specifically, this study investigated the efficacy of a bioprinted composite polycaprolactone (PCL)-alginate system, enriched with mesenchymal stem cells (MSCs) and gene delivery technology to drive mineralisation and bone formation *in vivo*. Non-viral gene delivery was achieved using nano-hydroxyapatite (nHA) particles to co-deliver two plasmid DNA (pDNA) vectors encoding for TGF-β3 and BMP-2; two genes which play a vital role in bone development and healing. These nHA-pDNA complexes were successfully printed within a RGD-modified alginate hydrogel, and co-printed with PCL