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Review

A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering

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ABSTRACT

The combined potential of hydrogels and rapid prototyping technologies has been an exciting route in developing tissue engineering scaffolds for the past decade. Hydrogels represent to be an interesting starting material for soft, and lately also for hard tissue regeneration. Their application enables the encapsulation of cells and therefore an increase of the seeding efficiency of the fabricated structures. Rapid prototyping techniques on the other hand, have become an elegant tool for the production of scaffolds with the purpose of cell seeding and/or cell encapsulation. By means of rapid prototyping, one can design a fully interconnected 3-dimensional structure with pre-determined dimensions and porosity. Despite this benefit, some of the rapid prototyping techniques are not or less suitable for the generation of hydrogel scaffolds. In this review, we therefore give an overview on the different rapid prototyping techniques suitable for the processing of hydrogel materials. A primary distinction will be made between (i) laser-based, (ii) nozzle-based, and (iii) printer-based systems. Special attention will be addressed to current trends and limitations regarding the respective techniques. Each of these techniques will be further discussed in terms of the different hydrogel materials used so far. One major drawback when working with hydrogels is the lack of mechanical strength. Therefore, maintaining and improving the mechanical integrity of the processed scaffolds has become a key issue regarding 3-dimensional hydrogel structures. This limitation can either be overcome during or after processing the scaffolds, depending on the applied technology and materials.

1. Introduction

To date, organ and tissue transplantation remains one of the most important while complex options in order to restore or enhance life expectancy. The most recent annual report prepared by the Scientific Registry of Transplant Recipients (SRTR) in collaboration with the Organ Procurement and Transplantation Network (OPTN) registered 112,905 patients in the USA awaiting transplantation at the end of 2011, while only 26,246 transplantations were performed [1]. If we keep the steady increase in life expectancy in mind, these numbers emphasize the shortage of organ donors [2]. In addition, diseases, infections and rejection of the tissue by the host often complicate transplantation [3]. To overcome these problems associated with transplantation, the last few decades, tissue engineering (TE) has grown as a new inter- and multi-disciplinary scientific field [4]. This discipline has rapidly emerged and combines the principles of engineering and life sciences. It holds as main objective the recovery, maintenance and improvement of tissue performance [4–6]. The European Commission on Health and Consumer Protection defines TE as the persuasion of the body to heal itself through the delivery, to the appropriate site, independently or in synergy, of cells, biomolecules and supporting structures [7].

Researchers will strive to fulfil those afore mentioned objectives through the utilization of isolated cells [8–11], tissue inducing substances [12–14] and/or scaffolds [3,4,6,15]. Although, conventionally, the application of a supporting scaffold is preferred in circumstances where the defect acquires certain dimensions. Post-processing cell seeding and maturation to tissue has therefore been implemented as a commonly applied TE strategy [15–19]. Expanding the cell population and maturation to tissue is performed in bioreactors, which can be described as devices in which biological and/or biochemical processes are manipulated through close control of environmental and process-bound factors such as pH, temperature, pressure, and nutrient and waste flow [20]. When working with low-water content polymers, post-processing cell seeding is the only available seeding mechanism. However, insufficient cell seeding and/or non-uniform cell distribution have been reported using this methodology [20,21]. There is thus a need for better and more uniform seeding principles. Enhancing the seeding efficiency can, among other, be accomplished by cell encapsulation

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strategies. This method requires a high-water content environment.

Hydrogels based on natural or synthetic polymers have been of great interest regarding cell encapsulation [22–37]. For the past decade, such hydrogels have become especially attractive as matrices for regenerating and repairing a wide variety of tissues and organs [7,12,33–92]. Depending on the hydrophilicity, they can absorb up to thousands of times of their dry weight and form chemically stable or (bio)degradable gels. Depending on the nature of the hydrogel network, 'physical' and 'chemical' gels can be distinguished. Hydrogels are called 'physical' when the network formation is reversible. In contrast to 'chemical' hydrogels, which are established by irreversible, covalent cross-links. Combinations of both physical and chemical networks can also be achieved, e.g. gelatine modified with methacrylamide groups [93].

The characteristic properties of hydrogels make them especially appealing for repairing and regenerating soft tissue [32,37–39,85–92,94–97]. One of the main disadvantages of processing hydrogels is the difficulty to shape them in predesigned geometries. This article will provide a detailed overview of the different rapid prototyping techniques that are compatible with hydrogel manufacturing and allow to accurately shape external and internal geometries. Since we did not find an article that summarizes the potential advantages and disadvantages regarding the processing of hydrogels with RP techniques, it is the purpose to highlight the advantages, but more importantly also the current limitations of the distinctive techniques, together with the respective hydrogels used so far.

In the first part, an introduction to scaffolding and basic concepts of scaffold-based and scaffold-free TE will be given. The next part handles hydrogel-friendly RP techniques used in scaffold-based TE. Finally, the implementation of RP technology in scaffold-free TE will be explained.

2. ECM mimetics: Current concepts

2.1. Scaffold-based vs. scaffold-free TE

From a cell biology perspective, 2D cell culture models only provide physiologically compromised cells induced by an unnatural environment [98], and the lack of a 3D structure will cause cells to form a random 2D mono-layer [17,19]. *In vivo*, cells are subjected to growth in three dimensions and complex cell–cell interactions. This observation encouraged a paradigm shift from conventional 2D cell culture models towards 3D microenvironments [99]. To obtain a more realistic understanding of cell–cell and cell–biomaterial interactions, Kirkpatrick et al. [100] proposed the use of co-culture models *in vitro*. Independent of the applied strategy, the ultimate goal of TE remains the same. Nevertheless, regarding the aspect of 3-dimensional cell migration, proliferation and differentiation behavior and requisites, one can distinguish two major premises. Currently, both of them are being heavily explored. The first one is based on the presumption that cells require a 3D biomaterial scaffold that closely mimics the corresponding extracellular matrix (ECM) [99,101]. In this approach, the biomaterial construct acts as a necessary cell guide and supporting template. The second one finds its roots in the hypothesis that cells have a considerable potency to self-organize through cell–cell interactions and is referred to as 'scaffold-free TE' [102]. While the former theory maximizes the role of a supporting structure as a cell guide and minimizes the potency to self-assembly, the latter reverses the importance of both contributions.

2.2. Scaffolds

Ideally, scaffolds can be seen as ECM biomimetic structures with three main objectives [17,18]: (i) defining a space that moulds the regenerating tissue; (ii) temporary substitution of tissue functions, and; (iii) guide for tissue ingrowth. It is clear that scaffold design should meet the needs of some basic requirements to be able to meet those objectives, including [3,15,17–19]: high porosity (preferably 100% interconnectivity for optimal nutrient/waste flow and tissue ingrowth); relevant geometry and pore dimensions (5–10 times the cell diameter); biodegradable with adjusted degradation time; maintaining the mechanical integrity during a prefixed time frame; it should have suitable cell–biomaterial interactions, and; be easy to manufacture. Adjusting the mechanical and degradation properties to the desired tissue is essential. Either enzymatic or non-enzymatic hydrolytic processes control the degradation profile. Specifically, TE requires biomaterials that provoke cell interactions (~bioactivity) [103] and as little as possible adverse body reactions (~biocompatibility) [104]. Control over the material bioactivity can be achieved by incorporating growth factors [105], enzymatic recognition sites [106], adhesion factors [94,107], or material modifications [106]. Material modification is a general term indicating either bulk modification [103,108] or surface modification [103,109,110]. Modifying the bulk properties is closely related to material biocompatibility, the physical and chemical properties covering the life-span of the implant [111], while varying the surface chemistry reflects on the initial cell/tissue–material interactions [111,112]. Fig. 1 illustrates schematically the complex multi-disciplinary interactions inherent towards scaffold fabrication. In the sub-science of scaffolding, both conventional and rapid prototyping (RP) techniques have been explored. Conventional scaffold fabrication setups include techniques such as particulate leaching [85,113–115], gas foaming [114–117], fibre networking [118,119], phase separation [120,121], melt moulding [122,123], emulsion freeze drying [124,125], solution casting [126,127], freeze drying [81,87,128] and combinations of those. Conventional/classical approaches are defined as processes that create scaffolds with a continuous, uninterrupted pore network. Nonetheless, they completely lack long-range micro-architectural channels [19]. Other reported disadvantages involve low and inhomogeneous mechanical strength, limited porosity and insufficient interconnectivity, inability to spatially design the pore

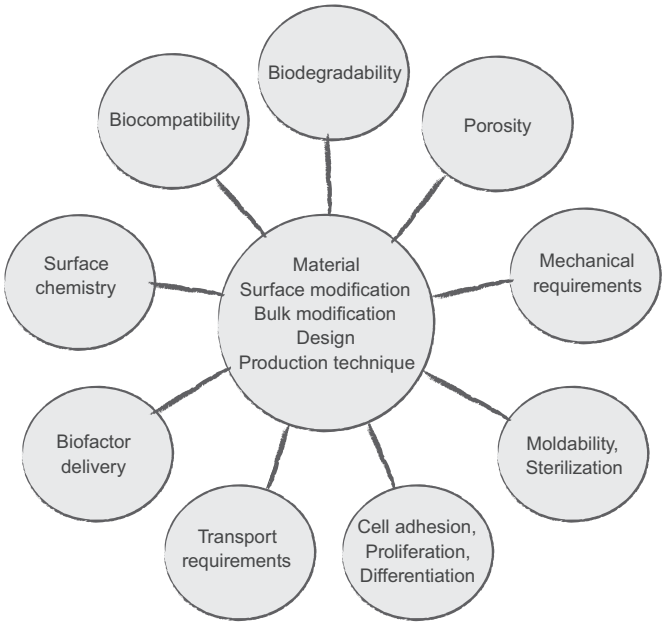

Fig. 1. Schematic illustration integrating the complex multi-disciplinary needs which determine the constraints for the ideal scaffold fabrication design.