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Review

Gelatin-Methacryloyl Hydrogels: Towards Biofabrication-Based Tissue Repair

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Research over the past decade on the cell–biomaterial interface has shifted to the third dimension. Besides mimicking the native extracellular environment by 3D cell culture, hydrogels offer the possibility to generate well-defined 3D biofabricated tissue analogs. In this context, gelatin-methacryloyl (gelMA) hydrogels have recently gained increased attention. This interest is sparked by the combination of the inherent bioactivity of gelatin and the physicochemical tailorability of photo-crosslinkable hydrogels. GelMA is a versatile matrix that can be used to engineer tissue analogs ranging from vasculature to cartilage and bone. Convergence of biological and biofabrication approaches is necessary to progress from merely proving cell functionality or construct shape fidelity towards regenerating tissues. GelMA has a critical pioneering role in this process and could be used to accelerate the development of clinically relevant applications.

Trends

In gelMA hydrogels, the inherent bioactivity of gelatin is combined with the tailorability of photo-crosslinking.

3D-generated tissue analogs need to be geometrically natural mimics that are biofunctionally and mechanically stable.

GelMA will accelerate the development of cell-laden biofabricated constructs and will have a pioneering role in their translation to clinically relevant applications.

Hydrogels and the Paradigm Shift to the Third Dimension

Over the past decade, cell culture research has witnessed a paradigm shift into the third dimension. 3D cultured cells behave differently compared with those cultured in monolayers (2D) and their responses better resemble those in the native tissue [1]. In this shift from the second to the third dimension, hydrogel-based approaches are driving current biomaterial research in tissue engineering. In tissue engineering, hydrogels are used that ideally resemble the natural extracellular matrix (ECM) to stimulate cells to form functional tissue with mechanical integrity to ensure survival of the graft upon implantation. While current synthetic hydrogels are often still too reductionist compared with biopolymers and, therefore, lack important biological cues [2,3], biological materials generally lack the necessary strength and precise mechanical tunability. In present-day biomaterial research, there is a strong need for a merger of both biologically active and physicochemically tailorable hydrogels [3]. Gelatin modified by methacryloyl (methacrylamide and methacrylate) side groups (gelMA) has recently gained increasing attention, because it satisfies the requirements of biofunctionality and mechanical tunability to a reasonable extent, particularly compared with other available hydrogel-forming biomaterials [4–9]. By using this 3D cell culture platform, not only is the natural extracellular environment represented, but it also provides the possibility to generate well-defined 3D tissue constructs [10–12]. In this respect, conventional 3D casting techniques for cell-laden hydrogels are replaced by advanced fabrication techniques. The emerging field of **biofabrication**

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Key Figure

Evolutionary Stages from 2D Cell Culture to the Development of 3D Tissue Analogs

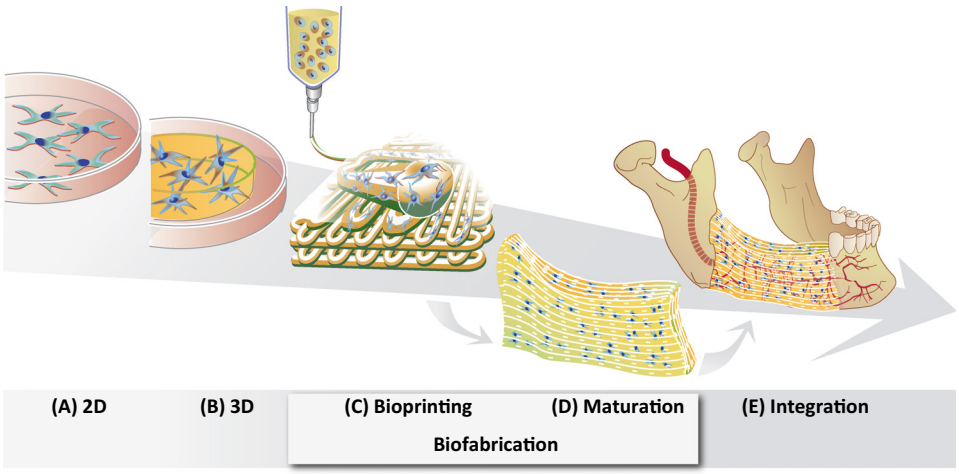

Figure 1. (A) 2D cell culture on plastic; (B) 3D cell culture inside hydrogel constructs; (C) bioprinting of 3D constructs; (D) biological maturation of the 3D bioprinted construct forming a tissue analog; and (E) implantation and integration of the tissue analog into the defect site.

(see Glossary) has as its aim the automated generation of biologically functional, hierarchical 3D constructs using living cells, bioactive molecules, biomaterials, cell aggregates, or hybrid cell-material and their subsequent maturation [13]. This advanced technology, which encompasses both bioassembly and bioprinting, allows for the generation of architecturally complex tissue analogs, which comprise a spatially organized assembly of various cell types potentially mimicking the native situation. This development of 3D tissue analogs reflects the evolutionary stages from cell culture in monolayers to 3D culture in disc-shaped hydrogels, to biofabricating 3D constructs undergoing biological maturation to ultimately repair a tissue defect *in vivo* (Figure 1, Key Figure).

In this review, we provide an overview of the uses of gelMA as a cell-encapsulating hydrogel, serving as a base material for a multitude of tissue-engineering strategies. We provide a picture of the diverse modifications of gelatin and its crosslinking systems, detailing the trends in gelatin-based biomaterial research, and the place of gelMA therein. In particular, we describe the use of gelMA in state-of-the-art biofabrication approaches to obtain complex tissue analogs, and we highlight the functional aspects of these developments. By doing so, we put into perspective the usefulness of gelMA-based engineered constructs in terms of the translational aspect of regenerative medicine.

Gelatin-Based Hydrogels for Cell Encapsulation

Gelatin is widely used in applications ranging from the food industry [14] to medicine and pharmaceutical processing [15]. In tissue engineering and regenerative medicine, gelatin is an attractive base material for engineering 'smart' hydrogels for drug delivery (e.g., [16,17]). Increasing interest in the use of gelatin in these fields stems from its various desirable features, including biocompatibility, biodegradability, low cost, and ease of manipulation [18]. Additionally,

Glossary

Biofabrication: 'The automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as micro-tissues, or hybrid cell-material constructs, through Bioprinting or Bioassembly and subsequent tissue maturation processes' [13].
Bioink: fluid or gel containing living cells to be used for printing of tissue constructs.
Endothelial colony-forming cells (ECFC): endothelial progenitors that are able to differentiate into functional endothelial cells. Although they are present in adult blood, they can be obtained with higher yield from umbilical cord blood for engineering endothelial networks or for coating the luminal side of vascular structures.
Habeeb method: method to determine the number of free amino groups in proteins.
Irgacure 2959: water-soluble, cytocompatible radical photoinitiator for the UV curing of unsaturated monomers and prepolymers.
Microfluidics: passive or active fluid handling or manipulation within micrometer-sized channels.
Micromolding: production of objects with micrometer-sized features within a mold.
Micropatterning: patterning of (bio) materials to control the fate and geometry of adhering cells.
Microtissue: hydrogels in the millimeter range with encapsulated cells that are used for 3D cultivation.
Mesenchymal stem cells or multipotent stromal cells (MSC): adult stem cells that can differentiate towards at least the osteogenic, adipogenic, and chondrogenic lineages. MSCs from human bone marrow aspirates are the gold standard human cell source used in tissue engineering and regenerative medicine of bone, fat and cartilage tissue.
Photo-crosslinking: covalent binding of molecules using light as an initiating system.
Poloxamer: a triblock copolymer with typical trade names 'Lutrol' or 'Pluronics'. It is used for defined printing processes for creating sacrificial layers or fibers in biofabrication approaches.
Polycaprolactone (PCL): a biodegradable and biocompatible