Uncovered

Epidermis recreation in spongy-like hydrogels

New opportunities to explore epidermis-like analogues

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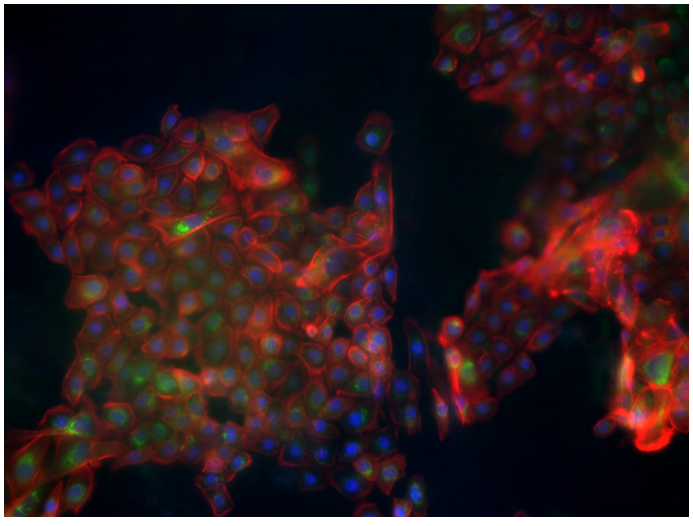

On the road to successfully achieving skin regeneration, 3D matrices/scaffolds that provide the adequate physico-chemical and biological cues to recreate the ideal healing environment are believed to be a key element [1–3].

Numerous polymeric matrices derived from both natural [4,5] and synthetic [6–8] sources have been used as cellular supports; nowadays, fewer matrices are simple carriers, and more and more are ECM analogues that can actively participate in the healing process. Therefore, the attractive characteristics of hydrogels, such as high water content, tunable elasticity and facilitated mass transportation, have made them excellent materials to mimic cells' native environment [9]. Moreover, their hygroscopic nature [10] and possibility of attaining soft tissues-like mechanical properties mean they have potential for exploitation as wound healing promoters [11–14]. Nonetheless, hydrogels lack natural cell adhesion sites [15], which limits the maximization of their potential in the recreation of the cell niche. This issue has been tackled through the use of a range of sophisticated approaches to decorate the hydrogels with adhesion sequences such as arginine-glycine-aspartic acid (RGD) derived from fibronectin [16–18], and tyrosine-isoleucine-glycine-serine-arginine (YIGSR) derived from laminin [18,19], which not only aim to modulate cell adhesion, but also influencing cell fate and survival [18]. Nonetheless, its widespread use is still limited by significant costs associated with the use of recombinant bioactive molecules.

A sequential but integrated processing methodology comprising the formation of a precursor hydrogel, its freezing, freeze-drying and re-hydration, was recently proposed by us to generate Gellan Gum (GG) cell-adhesive spongy-like hydrogels, retaining the attractive features of hydrogels, but with improved physical properties [20,21]. In fact, these structures permit overcoming the limitations of traditional hydrogels, such as reduced physical stability and flexibility, and handling restrictions. These spongy-like hydrogels are obtained from stable polymeric off-the-shelf networks upon a simple re-hydration procedure with any saline solution, including a cell suspension [20,21]. Thus, cells become entrapped and then adhere to the material without being subjected to any adverse conditions. Issues such as the reduced temperature window for viable cell encapsulation and homogeneous cell dispersion within the hydrogel structure are overcome with spongy-like hydrogels.

Spongy-like hydrogels' cell adhesive features are potentiated by the conjugation of a significantly lower water content, in comparison to precursor hydrogels, a microstructural rearrangement, characterized by pore wall thickening that occurs during processing, and pore size augmentation [20,21]. Their properties can be tuned by varying several conditions during the

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processing, including the amount and type of polymers used in combination with GG. Aiming at its application in the skin regeneration field, and being hyaluronic acid (HA) one of the major polysaccharides of skin ECM, we have further developed GG-HA (GG-HA) spongy-like hydrogels. In murine full-thickness excisional wounds, we demonstrated that human skin cell fractions were entrapped within the spongy-like hydrogel, hypothesizing that the recreated environment would enable cells self-organization *in vivo* [22]. In this work, GG-HA spongy-like hydrogel acted as a suitable supporting matrix for the transplanted cells during the early time points allowing them to contribute to the observed early wounds reepithelialization and neovascularization. Moreover, human adipose stem cells (hASCs) and human adipose microvascular endothelial cells (hAMECs), both from human adipose tissue, were entrapped in spongy-like hydrogels [23]. Our findings demonstrated a synergistic effect of the GG-HA structure, and adipose tissue cells were demonstrated, where microvascular endothelial cells and hASCs, obtained in high yields from an abundant and easily accessible issue, in combination with an off-the-shelf dried polymeric network, were able to meet quality regeneration parameters such as fast wound closure and re-epithelialization, a distinct dermal matrix remodelling, and improved neovascularization.

Based on these promising outcomes, spongy-like hydrogels have been used to recreate an artificial epidermis in order to attain improved *in vitro* models. The image shown on this issue's cover represents human keratinocytes (hKCs) cultured on top of GG-HA spongy-like hydrogels for 7 days. Keratinocytes were able to proliferate and re-arrange in a pavement-pattern manner upholding their typical cell–cell contacts. The cell cytoskeleton, clearly visualized after staining with phalloidin-TRITC (red), demonstrates this organization, while the expression of keratin 14 (green) confirms the maintenance of an early differentiation-associated phenotype.

Among the currently available epidermal models in the market, major obstacles such as limited cell number obtained from skin biopsies that can contain unidentified polymorphisms and terminally differentiated keratinocytes with reduced functionality, as well as reduced epidermal barrier properties [1] are still to be overcome. Thus, spongy-like hydrogels that have been shown to support the formation of a monolayer of keratinocytes at an earlier stage of differentiation represent a great opportunity to further explore the maturation of an epidermis-like analogue.

Further reading

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