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Chapter 8

Hyaluronan-Based Hydrogels as Functional Vectors for Standardised Therapeutics in Tissue Engineering and Regenerative Medicine

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Introduction: Hyaluronan for Tailorable Regenerative Medicine Products

Industrial Transposition and Clinical Translation of Standardised Complex Biologicals

Within design and development phases of novel medicinal products (e.g. combined advanced therapy medicinal products (cATMP), tissue engineering products (TEP), medical devices), primary therapeutic materials often require conjugation to ancillary inert, supportive, or potentiating delivery vehicles or scaffolds. For local delivery by transplantation of cellular materials for relatively soft tissue repair, or local diffusion of therapeutic components, hydrogels represent prime formulation choices (Hartmann-Fritsch et al. 2016; Fallacara et al. 2018). Finely tuned physico-chemical properties of hydrogels, intrinsic or environment-responsive, may play a preponderant role towards mediation, hindering, or stimulation of therapeutic potential borne by standardised cellular substrates or derivatives thereof. Optimised developmental

workflows may enable pragmatic regulatory classification of cell-based or cell-derived hydrogel preparations, addressing both current regulatory hurdles and economic constraints (Bertram et al. 2012; Heathman et al. 2015). In particular, sustainable and highly scalable industrial transposition capacity is a prerequisite in modern developmental approaches and must be a critical factor when implementing therapeutic cell sources, hydrogel components, and manufacturing methodologies (Huerta-Ángeles et al. 2018; Laurent et al. 2020b). Extensive industrial experience and hindsight have been generated around the use of versatile hyaluronan-based hydrogels, which allow arrays of functionalisation possibilities and tailoring to specific topical (i.e. epidermal, dermal) or musculoskeletal medical indications (e.g. topical rehabilitation preparations, viscosupplementation, volumetric supplementation). Numerous human and veterinary applications (i.e. medicinal, aesthetic, cosmetic) have been considered for hyaluronic acid (HA), serving as a backbone for quasi-infinite structural modification and physico-chemical property modulation (Fallacara et al. 2018).

Harnessing Regenerative Potentials of Biologicals for Optimised Clinical Benefits

In the present chapter, an overview of critical therapeutic product design considerations will be addressed, as well as application examples of hyaluronan-based preparations for cell therapy delivery, combined with various autologous or allogeneic optimised therapeutic cell sources (e.g. platelet-rich plasma, stem cells, banked primary foetal progenitors). Particular focus is set on specific parameters directly impacting the potential of considered formulations in terms of clinical translation and biological effectiveness. The necessity for robust designs, scalable and sustainable industrial workflows, and standardised therapeutic efficacy are outlined. Overall, it may be shown that systematic approaches of biotechnological processing are key in sourcing and manufacturing both raw materials for hydrogel constitution (e.g. biofermented hyaluronan) and homogenous primary therapeutic cell sources (e.g. foetal progenitor cells consistently and simultaneously derived from a single organ donation). Such cell sources may be differentially and parallely established from multiple tissues (i.e. dermis, cartilage, tendon, muscle, bone, lung, intervertebral disc, etc.), undergo bioprocessing through *in vitro* propagation under inductive conditions for multi-tiered cell bank establishment, eventually being valorised after large-scale GMP manufacturing and release of clinical cell lots or cell-derived cell-free materials (Hohlfeld et al. 2005; Laurent et al. 2020b). These concepts of cell sourcing and delivery method optimisation have enabled the generation of over three decades of clinical experience in Switzerland, with the use of foetal progenitor cells and hyaluronan in particular, and shall continue to tangibly contribute to improvement of patient health in cutaneous and musculoskeletal regenerative medicine applications in general (Grognuz et al. 2016).

General Properties, Physiological, and Therapeutic Roles of Hyaluronan-Based Hydrogels

General Physical and Chemical Properties of Hydrogels

Hydrogels have been generically and commonly defined as three-dimensional hydrophilic polymeric networks suited for relatively important hydric captation (i.e. water, aqueous solution, or biological liquids), while maintaining a dispersion state, rather than a solute state (Peppas et al. 2000). Statutory definitions of the hydrogel form are devoid of minima regarding relative water contents. Relatively low values of water content typically revolve around 10%–20% of initial dry weight values (Hoffman 2012). Hydrogels are typically constituted by aqueously suspended, chemically or physically cross-linked polymers, inherently displaying macroscopic similarities (i.e. soft texture, flexible material, and rubbery properties) with extracellular matrix (ECM) components or native soft biological tissues (Peppas et al. 2012). Synthetic hydrogel products may be designed to display stimuli-responsive behaviours, through specific controlled reactions to environmental modifications (e.g. pH, temperature, ionic strength, pressure) (Peppas et al. 2000, 2012; Thomas et al. 2007; Hoffman 2012). Hydrogel differentiation and classification criteria include polymer identity and type (i.e. physico-chemical properties), sourcing (i.e. natural, synthetic, hybrid), combinations (i.e. homopolymer, heteropolymer, or co-polymer gels), functionalisation (e.g.

chemical groups, peptides, biologicals), and cross-linking, as well as the overall composition (Peppas et al. 2000, 2012; Thomas et al. 2007; Hoffman 2012; Ahmed 2015). Such diversity and potential for specific modulation and property tuning have promoted hydrogels to the forefront of several disciplines, among which biomedicine (Hoffman 2012; Mandal et al. 2020).

Hyaluronan-Based Hydrogel Definitions

Biopolymers (i.e. human, animal, plant, or bacteria-derived) are often characterised by optimal cyto- and biocompatibility. This class of materials comprises the natural polysaccharide HA or hyaluronan, a ubiquitous constituent of all vertebrates and a key component in a variety of medical, pharmaceutical, nutritional, and cosmetic applications (Liao et al. 2005; Ahmed 2015; Fallacara et al. 2018). An overview of the sources and potential applications of hyaluronan and derived products is presented in Figure 8.1. The natural, non-sulfated, polyanionic, and unbranched (i.e. linear chains of disaccharide

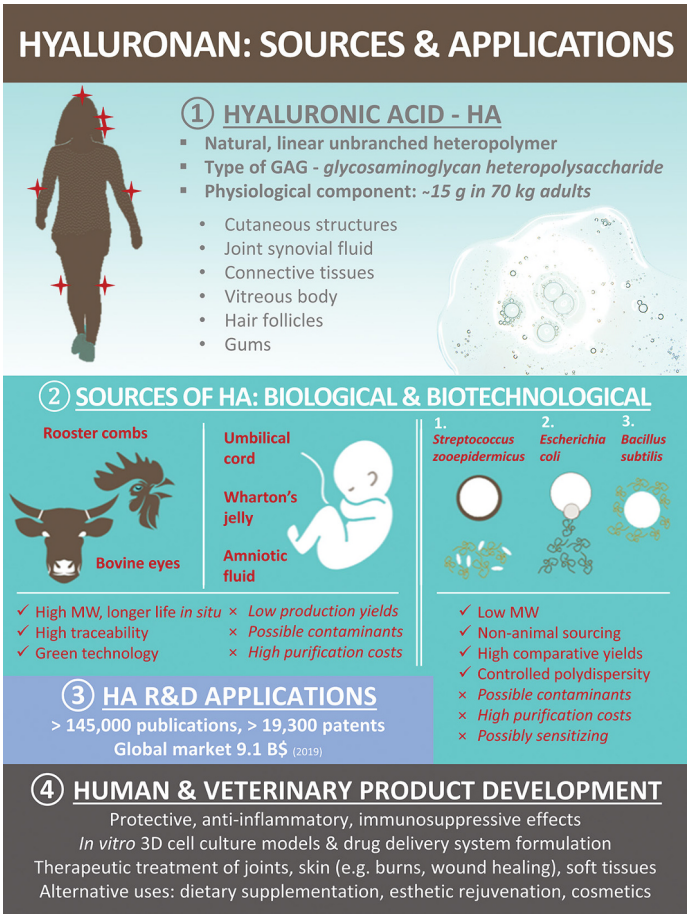

FIGURE 8.1 Overview of hyaluronan physiological anatomical locations, sources for therapeutic product development, current R&D statistics, and possible modern commercial applications. As a natural and ubiquitous physiological component, hyaluronan possesses diversified functions (e.g. structural, biomechanical, immunologic) (1). Historically, hyaluronan isolation and purification from food industry waste products were preferred for the obtention of large batches, while human gestational tissues and fluids have been studied and applied in some cases of burn wound treatment. Modern industrial processes have switched to bacterial fermentation for hyaluronan production, enabling obtention of homogenous and important yields of relatively lower molecular weight polymers. Respective advantages (i.e. ✓) and disadvantages (i.e. ×) are presented for biological and biotechnological sources of hyaluronan (2). The high interest around HA for R&D applications is attested by the considerable published literature, market statistics, and diversified modalities or indications for *in vitro* models and therapeutic products in development (3, 4). Market data was gathered from www.grandviewresearch.com (i.e. accessed November 2020).

units of N-acetyl-D-glucosamine and D-glucuronic acid linked by β-1,3 and β-1,4-glycosidic bonds) glycosaminoglycan (GAG) was coined as "hyaluronic acid" upon discovery by Meyer and Palmer in 1934, due to a mild acidic behaviour. Under physiological conditions, HA takes a polyelectrolyte form with associated ions, hence the alternative "sodium hyaluronate" or "hyaluronate" appelations, whereas "hyaluronan" refers to all possible forms of HA molecules (Dicker et al. 2014). Due to the presence of alcohol and carboxylic groups, HA is highly hydrophilic and polyanionic. Based on nuclear magnetic resonance spectroscopy (NMR), X-ray diffraction, and molecular modelling data, hydrogen bonds linking neighbouring disaccharides conform HA to an extended twofold helix secondary structure, with maximal overall molecular weights (MW) in the range of 10^8 Da (Liao et al. 2005; Fallacara et al. 2018). Overarching hyaluronan-based hydrogel network-related properties (i.e. viscosity and viscoelasticity) are directly related to MW, polymer concentration, and cross-link density.

Essential Physiological Roles of Hyaluronan and Related Bioengineering

Physiologically, hyaluronan is a highly hygroscopic ECM constituent found predominantly in the umbilical cord, connective tissues, synovial fluid, skin, and vitreous body of the eye, playing important roles in structural integrity and hydration maintenance. In cartilage tissues, hyaluronan represents up to two percent of total GAGs and plays essential lubrication and homeostasis functions (Kuiper and Sharma 2015; Fallacara et al. 2018). It is mainly synthesised by three transmembrane glycosyltransferase isoenzymes (i.e. hyaluronan synthases, HAS), and degradation is mediated by specific enzymes (i.e. hyaluronidases, HYAL) and reactive oxygen species (ROS) (Aruffo et al. 1990; Watterson and Esdaile 2000; Stern et al. 2007; Fallacara et al. 2018). Physiological turnovers are tissue-specific, wherein hyaluronan is renewed daily in the epidermis, once every three days in the dermis, and up to once every two days in cartilage (Fraser et al. 1993). In addition to water-retention and moisturising, specific and important hyaluronan functions may mediate and support cell migration, anti-inflammatory responses, angiogenesis, and tissue architectural organisation *via* interacting with collagen bundles. Such diversity in functions and effects may be partly due to the size variability of hyaluronan polymers. Natural location-specific decreases in HA content with ageing may play a role in diminishing tissue healing capacities. Additionally, in an inflammatory tissue context, HA degradation may be exacerbated, potentially mitigating the efficacy of various therapeutic products by restricting the residence time of the hydrogel in its original designed form. Nevertheless, due to important physiological functions of hyaluronan, high versatility, and emergence of modern physical and chemical processes, physiology-guided engineering has contributed to the democratisation of hyaluronan-based hydrogels in wide arrays of applications, specifically in the field of tissue engineering (Hoffman 2012; López-Ruiz et al. 2019).

Mechanisms Governing Physiological Hyaluronan Functions and Fate in the Skin

Hyaluronan synthesis in the skin by dermal fibroblasts and epidermal keratinocytes has been associated with CD44 receptors and shown to be under growth factor control (e.g. FGF, TGF-α/β, EGF, PDGF) (Aya and Stern 2014; Kavasi et al. 2017). Apart from structural purposes, additional cell environment control and protection roles have been attributed to hyaluronan, notably with the modulation of epidermal permeability and ECM composition, mediated by CD44 receptors (Fraser et al. 1997; Bourguignon et al. 2006). Physiological dermal hyaluronan is present in high molecular weight (HMW) form (i.e. around 10^6 Da), naturally producing cytoprotective, anti-angiogenic, and anti-inflammatory effects, as well as cytokine and growth factor bioavailability modulation, or induction of regulatory T cells (Dicker et al. 2014; Rayahin et al. 2015). Conversely, lysed hyaluronan in low molecular weight (LMW) forms (i.e. < 500 kDa) binds to toll-like receptors (e.g. TLR2 and TLR4), inducing the expression of pro-inflammatory genes, release of cytokines and chemokines by macrophages, and activation of dendritic cells and T cells (Gariboldi et al. 2008; Albano et al. 2016). Specifically, hyaluronan fragments smaller than 12 units induce an activated macrophage state, fragments between 8 and 32 units exhibit inflammatory and angiogenic properties, and the presence of fragments between 20 and 200 kDa reflects a state of tissular stress (Dicker et al. 2014; Rayahin et al. 2015). Physiological hyaluronan clearance is mediated by the liver and kidneys, wherein the normal blood half-life is of two to five minutes (Fraser et al. 1997). Along with effective size, polymer purity must also be taken into account to fully understand differential physiological effects of hyaluronan (Dong et al. 2016).

Pivotal Physiological Roles of Hyaluronan in Tissular Repair

In the context of skin lesions, hyaluronan accumulates at the repair site over three days to initiate tissue responses, maintains cell integrity, and finalises skin repair before being eliminated by HYAL enzymes after ten days (Mesa et al. 2002; Kavasi et al. 2017). Around 20%–30% of the hyaluronan turnover takes place locally in the skin, while the rest is eliminated by the lymphatic system. Interactions with specific receptors (i.e. CD44 and RHAMM) induce mitotic, migratory, and angiogenic responses (Dicker et al. 2014). Various specific hyaluronan polymer sizes establish and mediate the dialogue between damaged ECM and resident cells (e.g. fibroblasts, phagocytes), modulating both inflammatory and immune responses during tissue repair (Šafránková et al. 2010). Pivotal and specific roles of hyaluronan-mediated repair processes may be observed in developing foetuses, wherein high hyaluronan contents in the amniotic fluid prevent fibrosis and scarring (Nyman et al. 2013; Aya and Stern 2014). Specifically, longer persistence of hyaluronan may reduce collagen deposition and prevent scarring, leading to scar-free tissue repair in the foetus (Weindl et al. 2004). Such activities are drastically hindered in elderly organisms, functionally delaying or impairing the tissue repair process (Price et al. 2005).

Biotechnological Applications of Hyaluronan in Cell Culture Systems

Hyaluronan may be found in various modern cell culture systems or bio-printing workflows, wherein *ex vivo* cell culture is increasingly used for studies in the field of regenerative medicine (Murphy et al. 2013). Cell-supporting matrices are not limited to passive transport roles, but constitute essential microenvironments for cell signalling and proliferation (Bourguignon et al. 2006). Whereas monolayer cultures remain standard for *in vitro* cellular expansion and experimental systems, three-dimensional culture environments (i.e. static or dynamic) have been shown to optimally mimic native cell behaviours through physical scaffold provision (Turner et al. 2004). Natural and synthetic hydrogel sources have been characterised and proposed for cell culture, wherein the former types (e.g. collagen, hyaluronan, chitosan, alginate) are particularly biocompatible and functional for supporting cell viability, proliferation, and further development (Tibbitt and Anseth 2009). In view of tissue reconstruction, hyaluronan-based scaffolds are of great interest for mechanistic investigation of cell behaviour in the context of proof-of-concept establishment, as well as product biofabrication, as they mimic the natural composition and disposition of biological living tissues (Gurski et al. 2009; Jeffery et al. 2014).

Diversified Industrial Applications of Hyaluronan-Based Hydrogels

Numerous and diversified industrial applications have been proposed and investigated for native hyaluronan and related derivatives, non-exhaustively comprising drug delivery, cancer therapy, wound treatment, ophthalmology, arthrology, pneumology, urology, otolaryngology, odontology, or cosmetic and dietary applications (Fallacara et al. 2018). Specifically, drug carrier functions have been proposed for hyaluronan in prodrugs and liposomal, nanoparticle, microparticle, or hydrogel formulations, mediating controlled release, targeted, or enhanced pharmacodynamics of various therapeutic agents (e.g. anti-diabetics, steroids, anti-tumorals) (Kong et al. 2010; Chen et al. 2018; Fallacara et al. 2018). Vast clinical hindsight has been generated in orthopaedic applications, wherein arrays of marketed hyaluronan-based viscosupplementation products have been suggested as effective for managing symptoms and evolution of osteoarthritis (OA) and rheumatoid arthritis (Bowman et al. 2018).

Designing, Manufacturing, and Characterising Hyaluronan-Based Therapeutic Hydrogels

Hyaluronan-Based Therapeutic Hydrogels: Design Considerations

Intrinsic, modulable, and integrated characteristics or physico-chemical properties of hyaluronan (e.g. viscoelasticity, hygroscopicity, biocompatibility, tissue repair stimulation capacity) have favoured its adoption in a hydrogel form as an optimal bioengineering matrix, outperforming carbomers or sodium

alginate, with recognised benefits in cell therapies and regenerative medicine (Liao et al. 2005; Prestwich 2011; Guo et al. 2015; Mandal et al. 2020). Summarised advantages of this unique polymer are presented in Table 8.1. Numerous methods have been proposed for chemical modifications of native hyaluronan in view of cell encapsulation, drug delivery, volumetric or viscous supplementation, and related biomedical applications (Prestwich 2011; Schanté et al. 2011a). It was suggested that innovative hyaluronan derivatives constituted tools enabling vast improvement of industrial therapeutic developments, assorted to a specific claim that chemically modified biopolymers allow for exploitation of biological degradation pathways, unlike synthetic materials (Prestwich 2011; Schanté et al. 2011a). A synthetic workflow for hyaluronan-based combination product (e.g. cATMP) design and development is presented in Figure 8.2. It was further shown that design considerations (e.g. choice of MW, hyaluronan type and concentration) directly impacted therapeutic functionality (i.e. anti-apoptotic, anti-inflammatory, and cytoprotective effects) and clinical efficacy in a dose-dependent manner (Neuman et al. 2015). Specifically, and outside the controlled context of clinical trials, the restricted number of marketed ready-to-use cATMPs using hydrogel vectors may be partly explained by the low efficiency of cell viability maintenance, prompting the design of therapeutic kits comprising off-the-freezer cell sources to be extemporaneously suspended in an adequate hydrogel for improved clinical effectiveness (Schmidt et al. 2008; Prestwich 2011).

Background and Modern Processes for Hyaluronan Sourcing

Hyaluronan was discovered as a main viscous component in bovine eyes in 1934 (i.e. Dr. Karl Meyer, Ophthalmology laboratory, Columbia University) and was soon proposed for diverse therapeutic uses. Food chain availabilities and vast industrial potential led to the development and patenting of optimised extraction procedures for purification of hyaluronan from rooster combs (e.g. USA4141973A patent).

TABLE 8.1

Summary of Hyaluronan Properties and Related Benefits for Tissue Engineering. Adapted from Selected References

Property	Advantage	Comments	References
Natural ECM component	Complete biodegradability	High relative amounts of hyaluronan are found in the dermal layer of the skin	Lataillade et al. 2010
Non-immunogenic	Xenogeneic application in humans Complete biodegradability	Same polymeric structure of backbones across all species	Aya and Stern 2014
Non-allergenic	Generally safe for topical application or injection	May be free of endotoxins or be purified	Guo et al. 2015
High hygroscopicity	Maintenance of tissue hydration Facilitate the exchange of nutrients	Significant water retention Prevention of tissue dehydration and subsequent cell death May absorb 1,000 times its initial weight in water and attain 10,000 times its initial volume	Aya and Stern 2014 Guo et al. 2015
Viscoelasticity	Ideal biological lubrication	Viscosity variates as a function of oscillatory movements Rapid movements reduce viscosity by increasing elasticity, restoring deformation, and minimising cell distortion	Fraser et al. 1997
Non-adhesive	Ease of topical application and removal Easy monitoring	Painless wound dressing exchanges and patients have a pleasant feeling on the skin	Madaghiele et al. 2014 Shevchenko et al. 2010 Guo et al. 2015 Mahedia et al. 2016
Antioxidant	Protects the epidermis	Hyaluronan traps free radicals generated by UV rays	Weindl et al. 2004

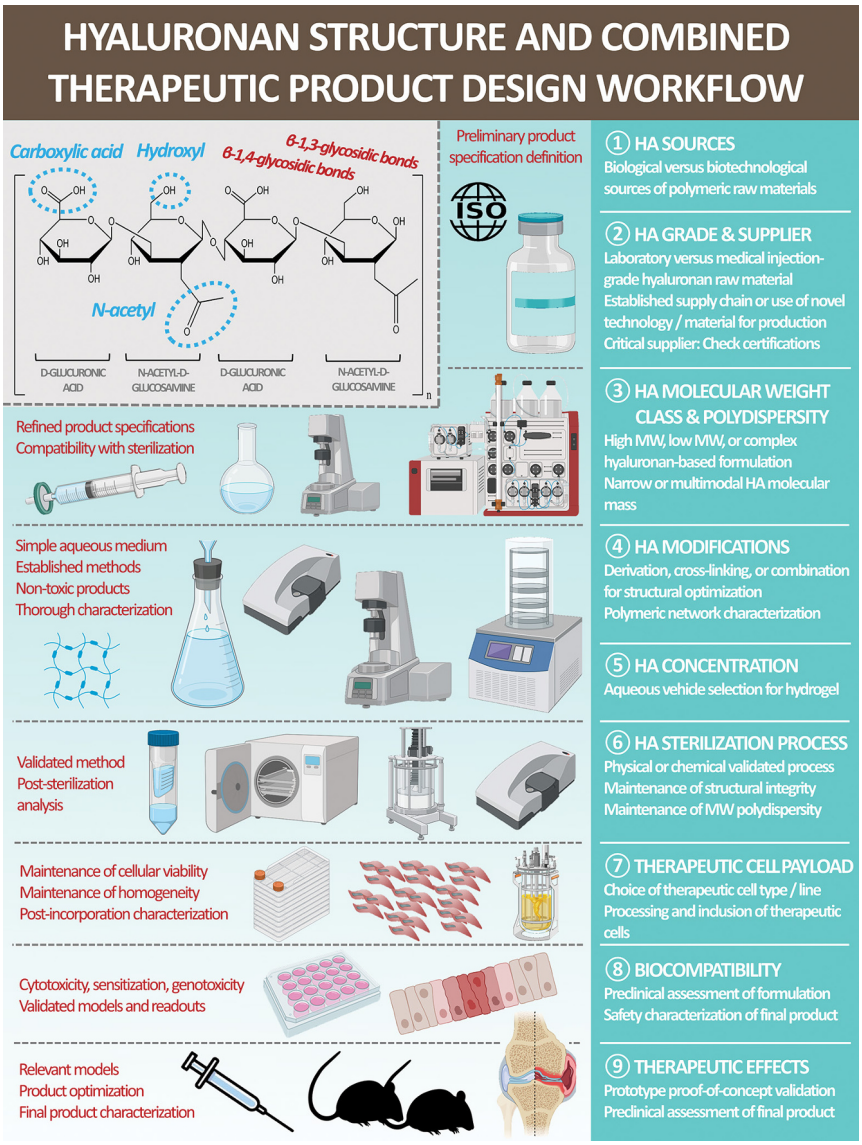

FIGURE 8.2 Chemical structure overview of hyaluronan (i.e. including modifiable functional groups) and comprehensive workflow for optimal hyaluronan-based combination product design and development. Supply chain considerations should ensure continuity of raw material availability. Based on development advancement (i.e. R&D or preclinical stage), various types and grades of hyaluronan may be used. Final product specifications partly depend on molecular weight class and polydispersity thereof (i.e. low or high molecular weight, combinations), which must be chosen appropriately for the considered sterilisation process, intended applications, and claimed effect. In particular, raw material specifications (e.g. analysis, acceptance criteria, storage) and product specifications (e.g. rheology, cohesivity, adhesivity, injectability, particle sizing) must be defined early in the development process. Hyaluronan structural modifications (i.e. conjugation or cross-linking) should be chosen to improve product parameters and effects, preferably using green chemistry and non-toxic reagents, while allowing characterisation (e.g. IR, NMR, SEC for process evaluation) and processing (e.g. lyophilisation). Hydrogel sterilisation should take into account the sensitivity of the polymeric network and of the therapeutic payload (e.g. cells or biologicals), and should be chosen among validated methods (e.g. ethylene oxide gas, steam, filtration, electron beam, gamma radiation) allowing post-sterilisation characterisation (e.g. rheology, SEC, NMR). Inclusion of therapeutic cells in the formulation must be performed while maintaining viability and homogeneity, with post-incorporation characterisation (e.g. injectability, cell viability at extrusion, stability testing). Following appropriate regulatory requirements, biocompatibility of products needs to be assessed after proper sample preparation and by using validated models. Finally, therapeutic proof-of-concept needs to be established on relevant models (e.g. *in vitro*, *ex vivo*, *in vivo*) for the intended product application.

The large red testosterone-responsive skin flap developed by roosters had been identified as the most prominent and ubiquitous tissue source (i.e. poultry waste product) for HMW hyaluronan (i.e. around 0.9 g of HA *per* kg of tissue, 4–6 MDa). Starting in the 1970s, hyaluronan was exploited for viscosupplementation of arthritic knees in the competitive horse industry to help the overworked equines reduce inflammation, and for veterinary ophthalmic uses. In human medicine, the original work of Dr. Balazs on the non-inflammatory fraction of hyaluronan extracted from biological tissues led to the development of various products. Non-exhaustive examples comprise Healon® for intraocular cataract surgery and lens replacement, Hylashield® for ocular pain and irritations, Synvisc® for viscosupplementation, and dermal viscoaugmentation solutions for depressed scars, fine lines, and wrinkles. Such marketed treatments have since then been used in hundreds of millions of patients world-wide (Selyanin et al. 2015; Fallacara et al. 2018).

Major interest from the pharmaceutical industry has continuously driven the development of hyaluronan sourcing from rooster combs and related products. The Swedish company Pharmacia benefited from the patents of Dr. Balazs to develop Healon®, before transferring the technology to Pfizer. Nevertheless, hyaluronan production continued in Sweden (i.e. Swedish white leghorn roosters), with such intensive output-driven breeding that chickens eventually could not maintain an upright head posture, due to excessive comb weight. Alternative poultry species have been bred in Northeastern USA farms and valorised by Genzyme for viscosupplementation and aesthetic medicine product development. Based on the extensive use of animal-sourced HA, it was demonstrated that processing of biological tissues was readily and scalably applicable in industrial settings (Kang et al. 2010; Kulkarni et al. 2018). Specifically, quantities of rooster combs reaching a metric ton could be processed by trituration and soaking, before the water-soluble fraction was filtered and treated with alcohol for obtention of hyaluronan in powder form. Alternative industrial hyaluronan sourcing methodologies comprise bacterial fermentation (i.e. around 6–10 g of HA *per* kg of culture, 1.5–2.5 MDa), avoiding the dependency to animal tissue supply (Agerup et al. 2005). Production yields are thereby greatly improved by hyaluronan derivation from *Streptococcus equi*, resulting in excellent immune tolerance and low risk of contamination by animal pathogens, and continue to be applied by many key suppliers (e.g. Lifecore Biomedical LLC, USA; Contipro a.s., Czech Republic; Givaudan International SA, Switzerland). Some concerns were raised around the potential pro-inflammatory effects of LMW hyaluronan and fragments thereof, wherein comparative studies have shown that endotoxin-free preparations are important to avoid inflammatory stimuli by the product (Dong et al. 2016).

Establishing Hyaluronan Raw Material Specifications and Characterisation Workflows

In view of industrial manufacturing for various types of products intended for human applications (e.g. medical devices, combination drug products), clear specifications and acceptance criteria must be defined for raw materials to be used in good manufacturing practice (GMP) production (Huerta-Ángeles et al. 2018). Raw material analysis using validated preparation and analytical methods should comprise macroscopic description, physico-chemical properties measurement, structural identification, stability testing, and impurities and biological contaminants analysis (Baeva et al. 2017). Specifications constitute an integral part of the product master file and define the acceptability criteria for inclusion in the production process. Based on predefined material specifications, adequately validated multifactorial characterisation (i.e. following Pharmacopoeial methods, ASTM standards, ISO norms) must be implemented to release hyaluronan batches. In addition to ensuring quality and adequation of raw materials, international standards and norms parallely enable holistic analysis of manufacturing processes from a risks and hazards point of view (e.g. ISO 14971 for medical devices). Applied to the design of a hyaluronan-based medical device for instance, such analysis should take into account the finished product and individual components, respectively, with a thorough assessment of sourcing and purity issues to exclude safety concerns (Huerta-Ángeles et al. 2018; Šafránková et al. 2018).

Hyaluronan Grade, Molecular Weight, and Polydispersity

Hyaluronan is a highly versatile biomolecule, as polymer MW has been shown to influence biological effects, therapeutic activity and efficacy, as well as product biodistribution, residence time, and clearance parameters. Differential effects of HMW versus LMW hyaluronan are presented in Table 8.2.

TABLE 8.2

Comparison of the Effects of HMW versus LMW Hyaluronan. Adapted from Gao et al. 2008, Pardue et al. 2008, Dong et al. 2016, and Fouda et al. 2016

HMW HA (>500 kDa)	LMW (<500 kDa)
Expressed in healthy tissues	Expressed in tissues under stress Produced under inflammatory processes where LMW and oligomers result from HMW HA degradation
Cytoprotector, influences the bioavailability of cytokines and growth factors	Increases the release of β-defensins by keratinocytes
Resolves inflammation by induction of regulatory T cells	Stimulates TLR receptors Induces the production of local TGF-β Activates dendritic cells and phagocytes
Puts the cells into quiescence (i.e. *via* CD44 receptor)	Stimulates angiogenesis (i.e. *via* CD44 and RHAMM receptors) Activates cytoskeletal remodelling (i.e. *via* RHAMM receptor) by stimulating cell migration

Native hyaluronan in healthy human synovial fluid exists in the form of HMW (i.e. over 1 MDa). In contrast, relatively LMW hyaluronan (i.e. < 500 kDa) has been shown to induce inflammatory responses in various cell types (Jiang et al. 2007). HMW hyaluronan is characterised by relatively high viscosity values, long biological retention times, and appropriate therapeutic effects (López-Ruiz et al. 2019). Due to the aforementioned considerations and modern development practices, products may contain hyaluronan of various MWs for tuning of physico-chemical properties and/or effect, while ultra HMW hyaluronan may be used to preemptively compensate for the degradation occurring during product heat-sterilisation. Initial and final polymer MW and polydispersity must in all cases be studied and validated. While laboratory-grade hyaluronan is suitable for design and product development steps, medical-grade raw material is required for final formulation and product registration and commercialisation phases. In any case, hyaluronan sourcing should comprise precise MW distribution parameters, definition of acceptable ranges for the specific considered application, and refining (e.g. using size-exclusion chromatography, flow field-flow fractionation) if necessary.

Vast Potential for Hyaluronan Derivation by Conjugation or Cross-Linking

Hyaluronan as a raw material may be obtained from different manufacturers in the form of a white powder, producing a highly viscous liquid (i.e. comparable to egg white) upon aqueous suspension, termed free HA, unmodified HA, or uncrosslinked HA, which is an excellent lubricant (Tezel and Fredrickson 2008). Soluble hyaluronan has been used in various clinical applications, such as viscosupplementation for OA, eye surgery, or wound healing, despite suboptimal intrinsic mechanical properties and rapid *in vivo* degradation. In view of chemical and biological properties alteration, hyaluronan may be modified on three functional sites presented in Figure 8.2, namely, the carboxylic acid group, the hydroxyl group, and the N-acetyl group (Fallacara et al. 2018). Structural manipulations may consist in grafting compounds to the hyaluronan backbone (i.e. conjugation, single chemical bond) or coupling of multiple polymeric chains (i.e. cross-linking, two or more chemical bonds) (Schanté et al. 2011a). Thereafter, hyaluronan derivatives may be classified as "monolithic," wherein the terminal form of the polymer does not allow new bonds to be created with cells or molecules, or as "living," wherein derivatives are able to form covalent bonds in the presence of cells, tissues, or molecules, thus allowing their incorporation prior to *in situ* gel formation by cross-linking (Prestwich 2011). By definition, living hyaluronan derivatives are therefore majorly favoured for combination product clinical applications or for preclinical uses, such as three-dimensional cell culture or formulation of therapeutic cells in view of *in vivo* delivery.

Overview of Hyaluronan Conjugation Processes

Exploiting the aqueous solubility of hyaluronan, various conjugation reactions have been proposed in water (Schanté et al. 2011a; López-Ruiz et al. 2019). Hyaluronan carboxylic acid modification in aqueous

solution with active amino groups *via* carbodiimides (e.g. 1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide, or EDC) has been widely used since almost 50 years and was well described (Danishefsky and Siskovic 1971). Activation of hyaluronan carboxyl groups with EDC in the pH range of 4–7 forms an active intermediate (i.e. O-acylisourea) which reacts promptly with primary amines (i.e. nucleophiles) to form stable amide bonds. To prevent rapid hydrolysis of the active intermediate, N-hydroxysuccinimide (NHS), 1-hydroxybenzotriazole (HOBt), or highly reactive N-hydroxysulfosuccinimide (Sulfo-NHS) may be added to the reaction, favouring the formation of an NHS ester intermediate. This intermediate is more stable and reacts slowly with primary amines to eventually form stable amide bonds. Depending on the specific manufacturing needs, reaction yields in water may be modulated by adjustment of pH, reagent quantities, and availability of amino groups (Schanté et al. 2011b; Maudens et al. 2018). To attain high relative reaction yields (i.e. >80%), the use of solvents such as dimethyl sulfoxyde (DMSO) or dimethylformamide in anhydrous conditions is necessary (Schneider et al. 2007; Schanté et al. 2011a). Hyaluronan carboxyl groups are furthermore known to be specifically recognised by HYAL-2 enzymes. This characteristic allows for degradation protection by means of chemical carboxyl conjugation (Aruffo et al. 1990; Schanté et al. 2011a).

Overview of Hyaluronan Cross-Linking Processes

To avoid *in vivo* solubilisation and subsequent expedited clearance, hyaluronan properties may be improved through cross-linking processes (Segura et al. 2005). Varying degrees of cross-linking enable the formation of polymeric hydrogel networks, conferring a uniform behaviour to the material, which might be considered as a chemical and physical barrier against enzyme and free radical degradation (Tezel and Fredrickson 2008). Specifically, rates of enzymatic degradation mediated by hyaluronidases are related to the degree of cross-linking of hyaluronan hydrogels, wherein higher degrees of cross-linking are associated with relatively improved stability. Additionally, cross-linking degrees along with polymer concentration greatly influence hydration balance values of specific hydrogels, defining the parameters for environmental water uptake and absorption. As an illustration thereof, the hydration balance in dermal fillers is generally achieved with 5.5 mg HA/mL of water and a typical degree of 4% cross-linking. Considering relatively higher hyaluronan concentrations (i.e. 20–24 mg/mL), the hydration balance values generally lead to important absorption of environing water by the product (Tezel and Fredrickson 2008).

Aqueous modification of hyaluronan hydroxyl groups by ether formation using epoxides was first reported in 1964, in strong alkaline conditions (i.e. pH 13–14) and 50°C (Laurent et al. 1964). To this end, 1,4 butanediol-diglycidyl ether (BDDE) is used for the reaction, mediating the opening of an epoxide ring to form ether bonds with hyaluronan hydroxyl groups. BDDE is a widely used cross-linking agent in the modulation of hyaluronan, due to accessibility of the reaction and absence of degradation product toxicity (Bogdan Allemann and Baumann 2008; Schanté et al. 2011a). Proposed applications consist in mixing and cross-linking HMW and LMW hyaluronan in various proportions using BDDE, thereby obtaining improved stability and resistance to degradation, optimal mechanical properties, and lower cytotoxicity (Xue et al. 2020). Exploiting hyaluronan hydroxyl groups and divinyl sulfone (DVS) as an alternative to BDDE confers the advantage of avoiding heating the reaction, yet intrinsic toxicity of DVS must be taken into account (Lai 2014). Modification of hyaluronan N-acetyl groups is not a first choice, since it requires preliminary modifications with deacetylation, carried out by hydrazinolysis, resulting in exposition of free amine functional groups. Furthermore, the conditions for deacetylation may then cause cleavage in HA backbones (Babasola et al. 2014).

Specifications for Therapeutic Hyaluronan-Based Hydrogel Products: Dermal Fillers and Viscosupplements

Specifications or target parameter ranges for therapeutic products based on hyaluronan strongly depend on the intended application and use, as well as expected clinical outcome, largely influenced by key physical properties of the polymer network. In the specific case of dermal filler hyaluronan hydrogels, specifications playing a preponderant role in design considerations comprise hyaluronan concentration, cross-linking, elastic modulus G', cohesivity, swelling, particle size, and extrusion force. With the introduction of BDDE as a cross-linking agent, the starting hyaluronan MW was rendered non-significant as a

parameter, due to the high increase in final product MW after cross-linking (Kablik et al. 2009; Molliard et al. 2018; La Gatta et al. 2019). For viscosupplementation products, considerations encompass the polymer MW distribution, gel rheological behaviour, lubrication capacity, and time of residence (de Rezende and de Campos 2015; Zheng et al. 2019). Raw material properties must be considered in order to fullfil the final product specifications, yet the specific manufacturing technologies and degrees of modification of the hyaluronan polymer may drastically impact product parameters and behaviour. Such processes may therefore be optimised, wherein the use of minimal BDDE reagent amounts to maximally cross-link the polymer with minimal reaction waste (i.e. pendant chains) representing a desirable manufacturing characterisation workflow (Kablik et al. 2009; Edsman et al. 2012; Molliard et al. 2018). Total degrees of modification can thus be defined as the addition of the cross-link percentage and the pendant chain percentage.

In order to enable dermal delivery of the product by injection (e.g. small-bore needles of 27-gauge and 30-gauge), the extrusion force must remain acceptable for human use. This parameter may be modulated by targeting adequate hyaluronan MW, viscosity, and polymer concentration, in order to eventually obtain a gel of appropriate viscosity at high shear rates. The incorporation of linear hyaluronan in a cross-linked formulation is an option to modulate polymer concentration while facilitating the extrusion. In this context, the elastic modulus G' represents gel ability towards volume recovery after deformation, with typical values laying between 10 and 10^3 Pa, most often superior in value to the viscous modulus G" (Pierre et al. 2015). Cohesivity further characterises the capacity to resist dissociation after implantation and may be partly assessed by compression testing.

Considering existing hyaluronan-based viscosupplementation product parameters, the mean values for polymer MW have been described between 0.5 and 10^6 Da. Polymer concentrations range from 0.8 to 30 mg/mL for linear, cross-linked, or hybrid network types in the hydrogel, which is usually conditioned in units of 0.5–6 mL (Bowman et al. 2018). For clinical application in OA patients, 21-gauge to 23-gauge needles are commonly used. Product rheological behaviours in terms of G' and G" (i.e. at constant oscillatory frequencies of 0.5 Hz and 2.5 Hz, simulating walking and running conditions, respectively) should be relatively elevated (e.g. 0.2–91.9 and 1.8–45.9 for G' and G", respectively) to improve joint lubrication (Bhuanantanondh et al. 2010). Improving joint residence time by derivation or addition of antioxidants is a key parameter in improving the clinical response, as short residence times are mediated by respective actions of HYAL enzymes and ROS (Stern et al. 2007; Conrozier et al. 2014; Maudens et al. 2018).

Finally, depending on the specifications of the individual raw material process and the product specifications established in the product master file, optimal storage conditions and instructions for use need to be established, given the relatively unstable nature of hyaluronan and derivatives. In particular, due to high hygroscopicity, materials and products should be stored in sealed original packages in clean, dry, and controlled-temperature environments. Depending on the nature and sensitivity of the additional therapeutic materials incorporated in the product, refrigeration and protection from light or air might be necessary (Huerta-Ángeles et al. 2018).

Sterilisation of Hyaluronan-Based Hydrogel Products

Sterilisation of hyaluronan-based products is complex, due to partial loss of viscosity, structural integrity, and function of the polymeric networks after exposure to high temperatures (i.e. >100°C), which typically occur during terminal sterilisation (e.g. autoclaving). Nevertheless, clinical application requires demonstration of product sterility. Alternative sterilisation methods have been proposed (e.g. ethylene oxide gas, electron beam or gamma radiation), yet each alternative presents potential detrimental effects as well (Huerta-Ángeles et al. 2018). Furthermore, an industrial manufacturing workflow is limited in the choice of validated sterilisation methods, or the task of validating a new method is to be undertaken. Therefore, when dealing with biologics and especially with therapeutic cellular materials or derivatives, aseptic processing may represent an alternative to endpoint sterilisation, in view of maintaining functional structures and related activity. Sterilisation by 0.22 μm filtration represents a good option depending on the concentration and the MW of the polymer. Based on sterilisation impact on the product, steam treatment and plasma radiation were shown to be efficient, in contrast with ethanol treatment or UV irradiation (Shimojo et al. 2015). Post-sterilisation validations of the physico-chemical integrity of the

product, such as MW determination, rheological characterisation, and measurement of other key properties, are needed to determine the extent of degradation or depolymerisation and safety of the product. Most hyaluronan-based products used for dermal correction or restoring synovial balance in small joints (e.g. Restylane®, Juvéderm®, and Ostenil®) are terminally sterilised in glass pre-filled syringes with a validated moist heat process in a pressurised autoclave. Specific sterilisation processes for medical devices are described in different normative documents (e.g. ISO 10993-7, ISO 17665-1, ISO 11737-2 and ISO 20857) (Huerta-Ángeles et al. 2018).

Characterisation Workflows for Hyaluronan-Based Hydrogels

Depending on the considered clinical application, claims for regulatory submissions, and related manufacturing and control requirements, many different aspects of HA hydrogels may be characterised and documented. The applied workflow may depend on the contents of the hydrogel or specific behaviours thereof (e.g. pH or temperature sensitivity such as particle thermoformation). The end-product may be analysed using orthogonal methods to determine the polymeric structure (e.g. IR, NMR, MS), degrees of substitution and cross-linking, size and morphology of particles (i.e. scanning electron microscopy, dynamic light scattering, nanoparticle tracking analysis), injectability, rheology (i.e. viscosity curves, elasticity, viscosity), stability (i.e. physical and under enzymatic or oxidative degradation), pH and zeta potential, *in vitro* drug release capacities (i.e. if an active pharmaceutical ingredient is incorporated in the hydrogel), cytocompatibility (i.e. *in vitro*, *ex vivo*, *in vivo*), etc. (Maudens et al. 2018; Miastkowska et al. 2020). Specific norms and reference standards are defined for evaluation of the different classes of products which may comprise hyaluronan-based hydrogels (e.g. ISO 10993: 2009 for medical devices).

Safety Evaluation of Hyaluronan-Based Products

For any type of product registration, adequate preclinical demonstration of safety (i.e. proof that the product is not pyrogenic, mutagenic, toxigenic, genotoxic, hemolytic, or immunogenic) must be provided. For medical devices, biocompatibility (i.e. assessment of cytotoxicity, sensitisation, and irritation/intracutaneous reactivity) and biodistribution must be documented within specific requirements, depending on the device type, category, residence time, and type of use (Huerta-Ángeles et al. 2018). *In vitro* acute cytotoxicity may be studied using adequate cell lines of cell types, within validated models coherent with the intended application (i.e. homologous with implantation site), and adequate methodology-specific sample preparation (e.g. extract-dilution method, test by direct contact, or indirect contact). Accepted readout methodologies for cytocompatibility testing comprise neutral red uptake (NRU), MTT (methyl thiazolyltetrazolium), XTT, resazurin assay, ATP concentration measurement, crystal violet staining, or DNA content measurement. For further preclinical safety evaluations, *in vivo* models (e.g. mice, guinea pigs, rats, rabbits, sheep) may be considered on an application-dependent basis, for evaluation (i.e. macroscopic description and histopathology) of the tissular response after product application. Such assays need to be devised based on projected product degradation rates, types of tissues exposed, and intended clinical exposure times, and may be used as safety demonstration before clinical testing of efficacy (Huerta-Ángeles et al. 2018).

Developing Hyaluronan-Based Hydrogels for Therapeutic Cell Delivery

Notwithstanding the considerations exposed hereabove and set forth for hyaluronan-based hydrogel development, which mainly revolves around the field of medical devices, additional parameters need to be taken into account for the sound development of biological combination products (e.g. cATMPs) for effective and safe delivery of therapeutic cellular materials. Such combination products are of high interest and have the potential to meet clinical needs, as they may act by exerting intrinsic additive or synergistic effects. Specific parameters and dimensions of combination product design and development are presented hereafter.

Therapeutic Cellular Materials for cATMPs

Vast arrays of tissues and cell sources may be considered as starting materials for therapeutic product development, among which platelet-rich plasma (PRP), stem cells, adult autologous stromal cells, or allogeneic foetal progenitor cells (Grognuz et al. 2016). Key considerations when developing combination products are the intended interactions and respective roles of the constituents. In this context, the hyaluronan-based hydrogel may be defined as an inert, cyto- and biocompatible vehicle for cell delivery, or as a functional and synergistic active ingredient. Two major parameters defining the formulation and conditioning of the final product are the extemporaneous mixing of therapeutic components versus the ready-to-use preparation, and incorporation of autologous or allogeneic cellular materials. The various designs may range from autologous and in-clinic preparation of the product (e.g. hyaluronan-suspended PRP) to serially manufactured formulations incorporating devitalised allogeneic cells or derivatives thereof (Abate et al. 2015; Wang et al. 2019). Overall, the key driver in designing the optimal cell therapy combination product is the maintenance or stabilisation of maximal therapeutic effects, which does not presuppose maintenance of cellular viability in all cases. Based on the intended application and therapeutic requirements, the hyaluronan hydrogel component may be tuned as described previously for various indications and modalities of administration, such as infiltration, soft-tissue partial replacement, viscosity enhancement and friction reduction, or complementary delivery of therapeutic materials to subcritical bone or cartilage defects.

In view of artificial tissue development, therapeutic cells are selected and designed to release specific biomolecules, and support matrices are tailored to provide intrinsic structural and regulatory signals to optimise tissue formation (Segura et al. 2005; Schmidt et al. 2008). Indeed, hematopoietic, embryonic, and mesenchymal stem cells physiologically reside in particular hyaluronan-rich microenvironments, remaining in a quiescent form with a low proliferation rate, in particular through interaction with essential hyaluronan receptors such as CD44 and RHAMM. Under control of PDGF, adult MSCs express CD44 and leverage interaction with extracellular hyaluronan to migrate towards regenerating tissues (Dicker et al. 2014).

Relative Proportions of Hyaluronan and Therapeutic Cells

Various parameters of hyaluronan polymeric networks may drastically qualitatively influence the cellular portion of the product. As an example, varying ranges of hyaluronan proportions and MW were used to suspend thermally-challenged murine fibroblasts, demonstrating an absence of clinically significant cytotoxicity in standard conditions, as well as relatively superior performance of hyaluronan versus carbomer and sodium alginate with regard to toxicity (Guo et al. 2015). Typical concentrations of LMW hyaluronan in topical combination treatments revolve around 0.2% (Gariboldi et al. 2008). With regard to the quantity of therapeutic cells *per* individual product dose, *in vivo* studies often report the use of 10^6 to 10^7 cells, yet we have previously shown that for foetal progenitor cells (FPC), clinical doses of 5×10^5 to 2.5×10^6 were sufficient for obtaining potent regenerative stimuli (Hohlfeld et al. 2005; Grognuz et al. 2016; Laurent et al. 2020b).

Cell Encapsulation within Hyaluronan-Based Hydrogels

Encapsulating therapeutic cellular materials within hydrogels may be beneficial for viability maintenance, targeted delivery, and protection from stress and degradation. The rheological properties of cell suspensions prior to hydrogel formation are fundamental to maintaining both cell viability and mutual adhesion during the encapsulation process. Two key process categories may be distinguished, with macro encapsulation (i.e. large quantities of cells in a large volume of product), and microencapsulation (i.e. cells introduced in small volumes of product). Micro-manufacturing techniques have been used to encapsulate cells with hyaluronan before a polymeric cross-linking step leading to hydrogel formation occurred. Excessive viscosity during cell suspension processes and product administration constitutes a major factor for viability loss, due to relatively high shear forces potentially damaging cell plasma membranes and preventing cell-cell contacts (Schmidt et al. 2008). Additionally, it was shown that for

cell microencapsulation in hyaluronan hydrogels, high polymer concentrations (i.e. 2%–15%) enable obtention of mechanical integrity of the microstructures, yet said higher amounts prove to be cytotoxic. An optimal concentration of 5% hyaluronan in water or phosphate-buffered saline (PBS) was therefore proposed for cell microencapsulation (Khademhosseini et al. 2006).

In order to improve cell viability in hyaluronan hydrogels, incorporation of dextran microspheres was proposed, due to the biodegradable, hydrophilic, and biocompatible properties of this polysaccharide, as well as its use in tissue engineering matrices. Human embryonic stem cells loaded in such formulations maintained an undifferentiated state, while conserving their full potency. Dextran microspheres provide an interface between the hydrogel and cells, synergistically promoting cell survival, activity, and tissue regeneration stimulation (Kim et al. 2010). It is of prime importance that controlled chemical reactions and hydrogel degradation products are not deleterious to the encapsulated cells, wherein chemical and mechanical properties of hydrogels are generally determined during the cell encapsulation process (Tibbitt and Anseth 2009). From a manufacturing point of view, hyaluronan derivation often requires relatively high temperatures and highly alkaline cross-linking conditions, which prevent the inclusion of living cells during hydrogel preparation. Modern workflows allow circumventing some of these potential pitfalls, with the rapid preparation of ready-to-use hyaluronan-based hydrogels under neutral conditions (Luo et al. 2000). Finally, cell spatial distribution within the hydrogel can be controlled by using bio-printing techniques, thereby accurately depositing the cells and encapsulating them in the hydrogel (Murphy et al. 2013; Madaghiele et al. 2014).

Viscosity Tuning in Hyaluronan-Based Cell Therapies

A precise definition of hydrogel characteristics is necessary to allow optimal product application and persistence (Jones and Vaughan 2005). Modulation of hyaluronan cross-linking degrees directly influences hydrogel dynamic viscosity values, expressed in units of Pa•s. To this end and in a specific tissue engineering application, we had previously compared various hyaluronan hydrogels with a hybrid objective of acceptable rheological properties and cellular viability maintenance (Grognuz et al. 2016). It was shown that for each additional unit of Pa•s, 0.3% of cell survival was lost, but a dynamic viscosity of less than 5 Pa•s was insufficient to appropriately maintain cells in suspension. It was found that an optimal balance between shear force applied on the cells and optimal viscosity for intended applications was obtained with the use of Ostenil® Tendon (i.e. 2% hyaluronan gel, TRB Chemedica SA, Switzerland) in tendon tissue engineering (Grognuz et al. 2016). It has been demonstrated that mechanical properties of cell carriers modulate both behaviour and phenotype of cells in a similar manner to biochemical cues. Particularly, cell migration requires a high degree of cellular spatiotemporal coordination and is therefore closely linked to mechanical properties of the carrier in which cells are distributed. Cells are subjected to high tensile forces in highly viscous environments, wherein the viscosity of the hydrogel may be proportional to the internal adhesion capacity and inversely proportional to migration speed. Therefore, viscosity is a limiting factor, as relatively high values may restrict cellular migration, while increasing adhesion strength. Furthermore, cell proliferation is also proportional to hydrogel viscosity to a certain extent (Ghosh et al. 2007).

Biochemical Signalling and Protein Transport within Hyaluronan-Based Hydrogels

For optimal effectiveness of biological combination products, the cell support matrix must provide an appropriate environment, relay signals directing or modulating cellular processes and tissue formation. Matrix surfaces ensure cell adhesion and migration, potentially influencing their survival or the ability to colonise surrounding recipient tissues. A determinant functional parameter is therefore the facilitation by the hydrogel of both influx and efflux of biological molecules, which may be modulated during polymer and gel synthesis by tuning porosity (Schmidt et al. 2008). A specific example thereof consists in using cryopolymerisation for cryogel formation, with the obtention of three-dimensional networks of interconnected macropores, yielding enhanced mechanical stability as compared to traditional hydrogels. Therefore, cryogels have been proposed for tissue engineering applications, as open pore structures

allow the transport of nutrients and oxygen to the cells, and conversely, the removal of cellular waste. Combination of the excellent biocompatibility of hyaluronan with the advantageous properties of cryogels has shown that adequate physiological environments may be created to support long-term cell cultures *in vitro* (Thönes et al. 2017). The advantages of macroporosity in hyaluronan-based matrices are further substantiated by the improved diffusion of encapsulated therapeutic cells towards recipient environing tissues (Burdick and Prestwich 2011).

Complex Hyaluronan-Based Hydrogels for Therapeutic Cell Delivery

Further modulation of therapeutic cell behaviour within hydrogels is attainable by introduction of cell adhesion sites on hyaluronan backbones or by adding adhesion proteins such as collagen, laminin, or fibronectin to the formulation. To this end, soluble hyaluronan is often mixed with collagen gels, in order to mitigate intrinsic mechanical limitations, reduce degradation rates, and restrict the relative quantity of retained water (Segura et al. 2005). A comparative study for vocal cord regeneration investigated the use of hyaluronan hydrogels, hyaluronan-collagen cogels, and hyaluronan-DCM (decellularised ECM). Hyaluronan alone and hyaluronan-collagen cogels were characterised by relatively limited biological activity for the proliferation support of encapsulated adipose stem cells. The incorporation of DCM provided an optimal environment for cell growth and differentiation, indicating that cogels based on hyaluronan and DCM would constitute promising support matrices for standard therapeutic cellular materials (Saddiq et al. 2009; Huang et al. 2016).

Self-Assembling Hyaluronan-Based Hydrogels for Optimal Responsiveness and Behaviour

We have previously reported the novel green chemistry manufacturing of biocompatible and biodegradable hyaluronan-based thermoresponsive hydrogels, cross-linked using a poly(N-isopropylacrylamide) (PNIPAM) polymer, named "HA Nano" or "HA Pearls" (Maudens et al. 2018). An overview of this specific technology is presented, along with advantages and potential therapeutic applications, in Figure 8.3. Appropriately linked to hyaluronan, *via* a cyclooctyne linker and a PEG spacer, PNIPAM enables the spontaneous formation of submicron spherical particles above a defined lower critical solution temperature (LCST). These 200-nm objects result from the self-assembly of PNIPAM moieties grafted onto the hyaluronan backbone which lead, at body temperature, to a unique nanostructured gel containing HA-PNIPAM spheres entangled in the gel network, without the use of chemical crosslinkers (Maudens et al. 2018). We have recently improved the aforementioned chemical production process in order to obtain finely tuned hydrogel biomechanical properties, depending on the chosen application (e.g. dermal fillers, long-lasting viscosupplementation, and vectors for cell therapies). Developed in the context of OA management and cutaneous delivery for improved injectability, persistence, and effectiveness, these materials (i.e. thermoresponsive gels) undergo reversible gelation through spontaneous nanoparticle formation at physiological temperatures (Brown et al. 1991; Schild 1992; Tan et al. 2009; Li and Guan 2011; Cooperstein and Canavan 2013). Major clinical benefits may be deployed by such novel formulations presenting thermoreversible, non-Newtonian pseudoplastic behaviours, enabling a facilitated injection of products and building high viscosity *in situ*, along with improved resistance against degradation (i.e. actions of HYAL and ROS) due to designed steric hindrance (Cilurzo et al. 2011; Maudens et al. 2018).

Hyaluronan-Based Hydrogel Stability and Degradation

In vivo biological activity of hydrogels is closely dependent on both microstructural (i.e. chemical composition, cross-linking density, etc.) and macrostructural (i.e. viscosity, degradation rate, etc.) characteristics, which may directly influence behaviours of encapsulated cells and indirectly promote or restrict integration thereof into recipient tissues. Particularly, cell migratory capabilities may rely on hydrogel degradation, as infiltration is inhibited by narrow dimensions of the polymer mesh (Madaghiele et al. 2014). Hyaluronan degradation in the hydrogel greatly depends on the concentration and exposure time to hyaluronidases (i.e. mainly HYAL-1 and HYAL-2). Due to intrinsic hyaluronidase production by various cell types including fibroblasts, the inclusion of live cells in hyaluronan gels negatively impacts long-term stability, with the introduction of "autophagic" components, adding to the effects exerted by recipient

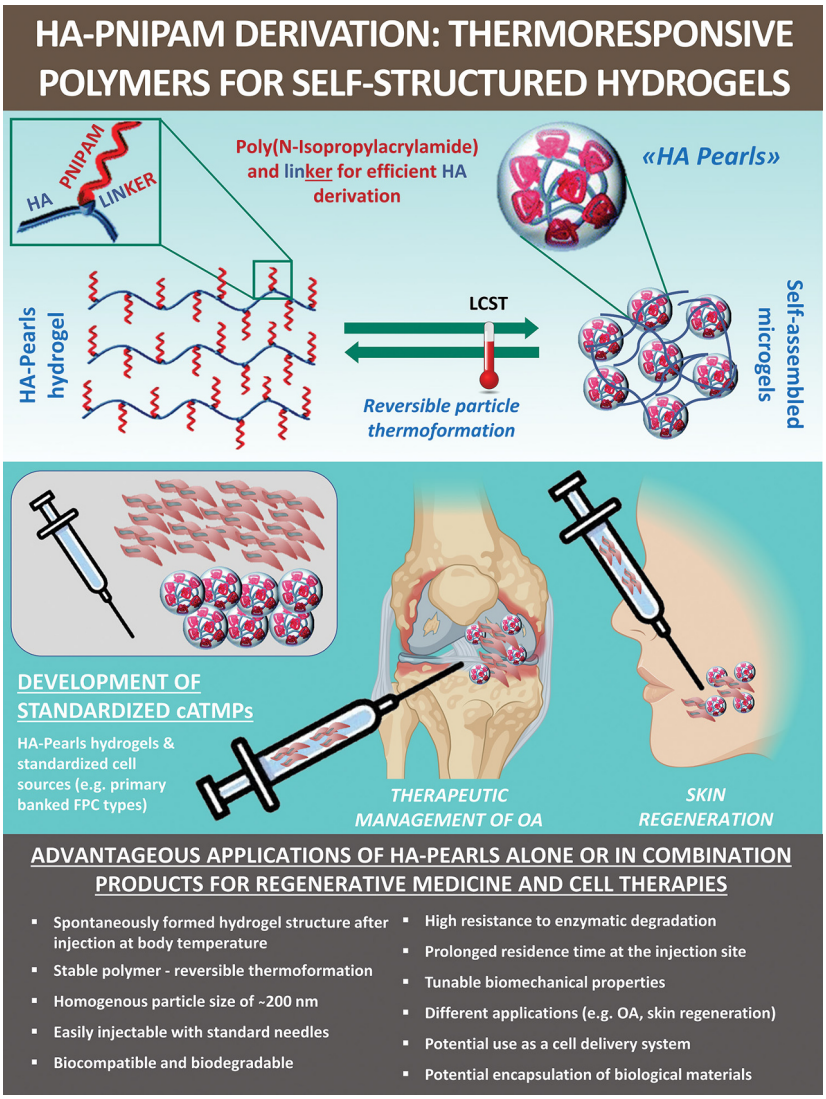

FIGURE 8.3 Overview of thermoresponsive hyaluronan-based hydrogels spontaneously forming particles at body temperature, assorted to potential applications and related benefits thereof. The combined use of appropriate linkers and PNIPAM for modification of the polymeric network confers unique properties to the formulation, namely, thermoresponsive and reversible self-formation of particles in the hydrogel. Chemical optimisation to obtain a specific lower critical solution temperature (LCST) inferior in value to body temperature but superior to ambient temperature enables simple injection of the hydrogel product, which develops specific and differential rheological properties *in situ* after administration. Appropriate conjugation of such thermoresponsive formulations with standardised cellular materials (e.g. FPCs or derivatives thereof) represents highly versatile novel therapeutic product development options for management of diverse conditions (e.g. OA, manifestations of ageing, etc.). Adapted from Maudens et al. 2018.

endogenous hyaluronidases. Nevertheless, it was demonstrated that despite complete and rapid enzymatic degradation of hydrogels with hyaluronidases and some macrophages, encapsulated cells could maintain viability (Khademhosseini et al. 2006; Madaghiele et al. 2014). Considering tissue engineering applications, ideal rates of hydrogel degradation should coincide with the rate of tissue formation. Insufficient degradation would indeed compromise remodelling, while rapid degradation would induce early resorption of the support matrix. It was hypothesised that for cutaneous applications, complete degradation of the hydrogel in seven days would allow optimal migration of angiogenic cells and induce tissue neo-vascularisation (Madaghiele et al. 2014).

Regulatory-Guided Hyaluronan-Based Biological Combination Product Development

Ambivalence in the current regulatory context of hyaluronan-based product development should be taken into account and is highlighted in the example of injectable products for OA, which the USA Food and Drug Administration (FDA) intends to reclassify as drugs rather than medical devices (https://www.federalregister.gov/documents/2018/12/18/2018-27351/intent-to-consider-the-appropriate-classification-of-hyaluronic-acid-intra-articular-products). While such products have historically been classified as drugs, medical devices, or both, recent literature interpretations by the FDA have put forward a primary intended purpose of treatment exerted through chemical action within the body in the case of OA, hence a potential intended use evading the scope of class III medical devices under USA regulations (Braithwait et al. 2016). Until now, medical device classification for hyaluronan-based OA viscosupplementation products was based on a mechanical action (i.e. lubrication, shock absorption by synovial liquid prostheses) as a primary intended purpose (Greenberg et al. 2006; Jahn et al. 2016). Notwithstanding, recent publications have supported chemical actions contributing to the analgesic, anti-inflammatory, and chondroprotective effects of hyaluronan-based orthopaedic products (Moreland 2003; Vasi et al. 2014; Richards et al. 2016; Altman et al. 2018). Therefore, as argued by the FDA, the primary intended purpose of such products would be attained by chemical means, by mitigation of the pathological condition itself. This aspect would be further substantiated by the long-lasting clinical therapeutic effects of hyaluronan on pain reduction (i.e. up to six months) in the knee. In view of such considerations, official advice to hyaluronan-based OA product manufacturers has been put forth by the FDA, guiding them towards preliminary classification and jurisdictional determination of products by means of pre- or request for designation (RFD) procedures, before submission of premarket approval applications (PMA).

Notwithstanding, various classifications of combination products may be considered for hyaluronan-based hydrogel preparations containing therapeutic cells or derivatives thereof, depending on the source and processing of the material (i.e. autologous or allogeneic, culture-expanded or minimally manipulated), intended application and modalities (i.e. topical or invasive), and defined state of the biological raw materials (e.g. living cells, devitalised cells, fractions, conditioned medium, etc.) (Laurent et al. 2020b). Although some specific applications may be defined as medicalised cosmetics, most applications covered by various legal and regulatory frameworks separate the products described herein in specific categories, such as cATMPs and class III medical devices, depending on the mode of action of the product (i.e. principal or ancillary effect of the biologicals) and the claimed effect (Racchi et al. 2016). Due to highly different requirements and possibilities for the aforementioned regulatory classifications, close attention must be paid and risk mitigation must be performed at the beginning of the product development phase, in order to ensure compliance with applicable regulations in the intended markets.

Tissue Engineering Clinical Applications of Hyaluronan-Based Hydrogels for Standardised Cell Therapies

Clinical Hindsight and Advantages of Hyaluronan-Based Therapeutics

Hyaluronan has been extensively investigated as a vehicle for drug delivery, whether considering ophthalmic, nasal, pulmonary, parenteral, or topical applications (Brown and Jones 2005). In topical applications, hyaluronan appears to increase the residence time of other agents, as during an inflammatory state, CD44 and RHAMM receptors are up-regulated and favour hyaluronan binding (Weindl et al. 2004). Due to ECM-mimicking properties of hydrogels, along with optimal biocompatibility, biodegradability, and applicability to support encapsulation of cells or growth factors, such preparations have been widely used in various pharmaceutical fields. Even though hyaluronan was first discovered in 1934, the first clinical application was reported in 1968 for the treatment of burn victims (Fatini et al. 1968). Since then, multiple facets of human and veterinary medicine have benefitted from its application, proven to be effective in various fields such as ophthalmic and aesthetic surgery, pulmonary and rheumatological pathologies,

development of cosmetic products, as well as skin regeneration (Juhász et al. 2012). Various hyaluronan-based biomaterials have been developed for the controlled release of growth factors or cytokines which induce cell proliferation. The immunomodulatory action of hyaluronan may be beneficial, through the increases of tissue regeneration capacities in burns, surgical epithelial wounds, or chronic wounds (Fouda et al. 2016).

Topical Management of Burns and Wounds using Hyaluronan-Based Biological Therapies

As a non-adherent material allowing generation of moist and sterile "*in vivo*-like" environments, hyaluronan hydrogels allow for rehydration of tissues and absorption of skin lesion exudates in a reversible manner, depending on environmental stimuli (i.e. temperature, pH). Various hyaluronan-based products have been proposed for the topical management of burn wounds, including cell-free (e.g. Hyalomatrix®, Hyalosafe®, HYAFF®-11, Ialugen®) and cell-laden constructs (e.g. Hyalograft 3D™, Laserskin™) (Turner et al. 2004; Tezel and Fredrickson 2008; Shevchenko et al. 2010; Longinotti 2014; Dalmedico et al. 2016). Clinical studies have confirmed the benefits of such constructs in the treatments of burn victims (Harris et al. 1999; Price et al. 2007; Gravante et al. 2010; Voigt and Driver 2012; Fino et al. 2015). Hyaluronan hydrogels have been characterised as moderately beneficial for topical delivery of therapeutic materials, depending on the extent and gravity of cutaneous lesions, with variable tissue penetration capacities, wherein a MW of 100 kDa enabled optimal passage through the disrupted skin barrier (Mesa et al. 2002; Witting et al. 2015). Qualitatively, hyaluronan hydrogels provide comfort during application, with a refreshing sensation and soothing effect which may contribute to significantly alleviate pain (Jones and Vaughan 2005). Structurally, hyaluronan hydrogels mimic native ECM and promote maintenance of tissue hydration and oxygenation *via* water retention, detritus and pathogenic microorganisms trapping, and cell protection by creating a physical barrier (Madaghiele et al. 2014; Guo et al. 2015). Their use as a supporting matrix for wound treatment may be complemented by the combined use of therapeutic cells, which may be encapsulated within the hydrogel, wherein culture conditions within such three-dimensional environments have been shown to be optimal. In addition to intrinsic biocompatibility and biological activity, natural hyaluronan confers various benefits in tissue engineering workflows, such as ECM remodelling chaperoning or the promotion of cellular functions of both therapeutic transplanted cells and recipient endogenous cells (Khademhosseini et al. 2006; Dicker et al. 2014; Thönes et al. 2017). Additionally, cell encapsulation within a biomaterial may potentially reduce inherent immunogenicity of therapeutic materials (Schmidt et al. 2008).

Various alternative or functionalised hyaluronan-based formulations may be considered for conjugation to therapeutic cells, notably with the inclusion of classical drugs, peptides, or other biological products (e.g. vitronectin) for burn repair and re-epithelialisation stimulation (Brown and Jones 2005; Xie et al. 2011). Storage and controlled release of bioactive factors, zinc, or silver sulfadiazine by hyaluronan gels may present therapeutic interest in complex chronic ulcer or burn cases requiring long-term anti-inflammatory effects and anti-microbial management (Koller 2004; Costagliola and Agrosì 2005; Lee et al. 2008; Juhász et al. 2012; Su et al. 2014; Das and Baker 2016). Intrinsically, hyaluronan was shown to elicit anti-bacterial responses (i.e. stimulation of β-defensin 2 by LMW hyaluronan), immunomodulatory, and anti-inflammatory effects (i.e. Ki-67 proliferation antigen expression reduction and CD44 receptor blocking by HMW hyaluronan) (Mesa et al. 2002; Lataillade et al. 2010; Schlesinger and Rowland Powell 2014). Cell-laden hyaluronan hydrogels were shown to accelerate tissue repair, re-epithelialisation, and neo-vascularisation in diversified applications (Neuman et al. 2015).

Hyaluronan-Based Products for Effective Management of OA using Autologous PRP

Relatively simple and standardised cell therapies, which have recently been democratised for various applications, consist in extemporaneous concentration of patient platelets and injection in appropriate vehicles such as hyaluronan. Despite the widespread use of bedside kits for PRP preparation (i.e. contained blood processing materials and a centrifuge), it should be noted that appropriate GMP

manufacturing is required. Such standardised autologous approaches have been proposed and studied for topical rejuvenation of the skin, management of subcritical tendon injuries, or OA (Ulusal 2017; Tan et al. 2021). Encouraging results have been reported for the use of PRP-yielding hyaluronan gels in OA patients which had previously not responded optimally to hyaluronan injections alone (Saturveithan et al. 2016; Renevier et al. 2018; Yu et al. 2018; Pereira et al. 2019). Specifically, PRP-hyaluronan injections were shown to be safe and to significantly delay the need for replacement surgeries and arthroplasties in suffering OA patients (Altman et al. 2015; Honvo et al. 2019; Ong et al. 2019).

Effective Modulation of Cartilage Repair by Hyaluronan-Based Therapies

OA remains a major clinical challenge, often refractory in its degenerative progression, which might synergistically benefit from combination products containing hyaluronan-based vehicles and therapeutic cell sources (Sekiya et al. 2012; Li et al. 2018). Hyaluronan-based formulations (i.e. HA Pearls) were shown to exert significant intrinsic effects on pro-inflammatory cytokine levels in a preclinical OA model (Maudens et al. 2018). For combination products, most preclinical experience has been gathered around the intra-articular use of stem cells for tissue repair stimulation, wherein hyaluronan mediates both the repair process itself and the function of therapeutic cells (Wang et al. 2020). Specifically, control of inflammation and chaperoning of exogenous and endogenous cell repair processes have been attributed to such combination products, wherein the level of hyaline cartilage restoration is dependent on the effective chondrogenesis (Ha et al. 2015). In this context, cartilage FPCs have been shown to present robust advantages for tissue repair or regeneration promotion after local delivery (Darwiche et al. 2012; Choi et al. 2016; Park et al. 2020). Whereas cartilage cell therapies have often been limited by the surgical approach or choice of the scaffold, subcritical defects might largely benefit from designed cell-laden hyaluronan hydrogels. Recent clinical trials for OA have been launched, confirming the safety and probable functional benefits of cells from foetal sources (Lee et al. 2018, 2020).

Importance of Standardised Cell Sources in Regenerative Medicine Products and Therapies

The task of defining novel biological raw materials or active pharmaceutical ingredients (API) is burdened by specific stringent prerequisites and quality considerations, in view of regenerative medicine product development or study of cell therapies (Platt et al. 2020; Yamazaki et al. 2020). Vast arrays of heterogenous tissue or cell sources have been proposed, comprising autologous, allogeneic, or xenogeneic materials of various developmental stages to be further processed by culture expansion, thereby introducing potential inherent multifactorial complications in development processes (De Buys Roessingh et al. 2013). Such proposed sources have non-exhaustively comprised embryonic stem cells (ESC), adult stem cells [i.e. adipose stem cells (ASC), bone marrow-derived mesenchymal stem cells (BM-MSC)], neural stem cells (NSC), limbal stem cells (LSC), hematopoietic stem cells (HSC)], endothelial progenitor cells (EPC), foetal progenitor cells (FPC), umbilical cord cells, neonatal foreskin cells, platelets, placenta, and amniotic fluid cells (Vertelov et al. 2013; Heathman et al. 2015; Mount et al. 2015; Muraca et al. 2017; Li and Maitz 2018; Sacchetti et al. 2018; Jayaraj et al. 2019; Torres-Torrillas et al. 2019). Imperious biological, logistical, sustainability, and clinical considerations synergistically contribute to the optimisation of cell source selection and product design phases. Harnessing of appropriate therapeutic cell sources for bioprocessing and product formulation presupposes safety and consistency of considered materials, traceability and sustainability of the source, high inherent expansion potential, and adaptability to clinical delivery methods often based on bioengineered scaffolds (Monti et al. 2012). Many cell sources are technically demanding and require specific processing or strong biochemical cues, wherein complex requirements often delay or restrict the potential for product development (Heathman et al. 2015). Potential barriers might arise with slow cellular proliferation, rarity of donors, unstable potency, low stability, or high propensity towards communicable disease transmission (Rayment and Williams 2010; Ratcliffe et al. 2011; Abbasalizadeh and Baharvand 2013; Hunsberger et al. 2015).

Facilitated Translational Research using Standardised FPC Sources for Complex Biological Products

Cell source selection is paramount for sound translational development and implementation of cellular therapy products. Iterative optimisation of standardised cell selection workflows has allowed to select allogeneic primary FPC types as potent candidates for cell therapy development, in addition to their recognised role as vaccine production substrates since the 1960s (Hayflick et al. 1962; De Buys Roessingh et al. 2006; Mirmalek-Sani et al. 2006; Larijani et al. 2015; Kim et al. 2018). A detailed overview of primary FPC isolation and biobanking is presented in Figure 8.4. Adequately isolated, such cell sources are advantageous in many aspects, such as a defined and tissue-specific phenotype, low *in vitro* growth requirements (i.e. independence from growth factors), high cytocompatibility and tolerance to oxidative stress, extensive lifespans, low immunogenicity, and high stimulatory potential (Cass et al. 1997; Quintin et al. 2007). Robust multi-tiered banking enables scalable good manufacturing practices (GMP) production, wherein sterility, safety, identity, purity, potency, stability, and efficacy may be demonstrated under stringent quality standards for cATMP development. Additionally, generation of large stocks of allogeneic cellular raw materials allows for drastic minimisation of product clinical availability delays. Foetal tissues or derived FPCs were used in various clinical applications, notably neurology (i.e. Huntington's or Parkinson's disease, strokes, spinal cord injuries), haematological or metabolic disorders, or liver failure (Touraine et al. 1993; Freeman 1997; Rosser and Dunnett 2003; Montanucci et al. 2013). Over the past three decades, we have reported notable clinical advances in the field of allogeneic FPC therapy, notably for managing burns and chronic ulcers, as well as arrays of musculoskeletal potential applications (Hohlfeld et al. 2005; Ramelet et al. 2009; Laurent et al. 2020b).

Swiss Foetal Progenitor Cell Transplantation Program for FPC Sourcing and cATMP Development

Within current regulatory frameworks for cell therapy product development, transplantation programs are well adapted for traceable procurement of safe and effective biological samples serving for standardised cell isolation procedures. Practical and sustainable designs for cell sourcing workflows are primordial for cell therapy or tissue bioengineering product development, guaranteeing homogeneity, consistency, robustness, and efficacy of therapeutic materials (Kent and Pfeffer 2006). Transplantation programs offer solid bases for optimised material procurement and therapeutic cell type establishment, ensuring regulated and traceable processing within. Swiss foetal progenitor cell transplantation programs were initially designed in the 1990s in Lausanne for the controlled cell banking of FPCs in view of tissue engineering development. Such federally registered programs are governed by organ transplantation laws and approved by ethics committees, public health authorities (i.e. FOPH, FDA, TFDA, and PMDA to date), and eventually by the national therapeutic products agency (i.e. Swissmedic). Multidisciplinary collaboration and exhaustive descriptive documentation enable the identification of qualifying donors after voluntary pregnancy terminations, in view of safe and standardised FPC derivation (Laurent et al. 2020d). Repeated serological testing (i.e. EBV, HIV, HTLV, hCMV, HHV, HSV, HBV, HCV, HPV, West Nile virus, syphilis) allows for screening of donors, before differential and simultaneous FPC isolation may take place. To this end, primary diploid cell culture initiation may be performed, after a single organ donation, on arrays of tissues such as skin, cartilage, tendon, bone, muscle, intervertebral disc, or lung, using enzymatic or mechanical methods for rapid, safe, sustainable, and efficient cell bank generation (Quintin et al. 2007; Laurent et al. 2020b). Subsequent multi-tiered cell banking optimised workflows (i.e. sub-tiering cryopreserved cell lots in Parental, Master, Working, and End of Production Cell Banks) may be transposed to industrialised processing, providing starting materials for decades of research and clinical applications (Laurent et al. 2020c). High stability and consistency of such scalable cell sources present optimal fits for modern safety and quality-driven regulations governing therapeutic product development (Hunsberger et al. 2015). A single qualifying organ donation may, therefore, for each considered tissue, simultaneously yield sufficient starting material for eventual GMP manufacturing of $> 10^8$ therapeutic product units of relatively small effective doses (i.e. 5×10^5 to 2×10^6 cell equivalents *per* dose on a

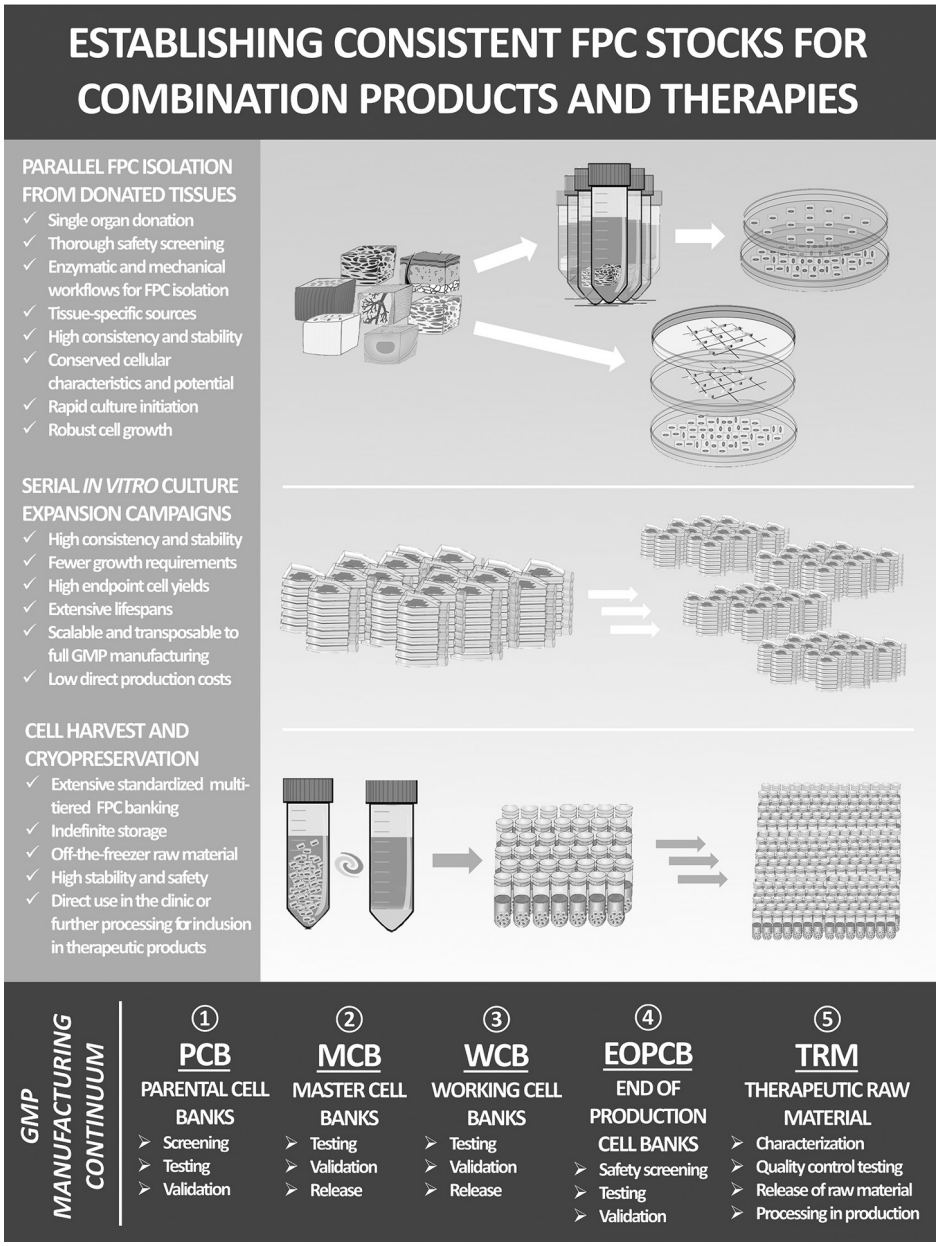

FIGURE 8.4 Schematic overview of standardised FPC isolation and banking methodologies for combined regenerative medicine product formulation. High efficiency may be attained by harvesting several tissues after a single organ donation. Subsequent parallel processing of biopsies (i.e. enzymatic and mechanical culture initiation) enables the rapid establishment of extensive and consistent Parental Cell Banks (PCB), which may be cryopreserved for long-term storage. Simple *in vitro* culture conditions and intrinsic robustness of primary FPC types allow for multi-tiered biobanking and optimal valorisation of therapeutic material stocks. In such GMP workflows, Parental Cell Bank vials serve for successive establishment of Master (MCB), Working (WCB), and End of Production Cell Bank (EOPCB) stocks, allowing for a thorough evaluation of safety and quality of manufactured batches. Selection of adequate lots thereafter enables optimal usage of cell banks for processing of therapeutic raw materials (TRM) to be used in combination products.

cell type-specific basis) (Hohlfeld et al. 2005; Darwiche et al. 2012). Applicable bioprocessing workflows additionally allow for the implementation of safety screening or testing and quality controls (e.g. assays to detect mycoplasma, viruses, prions, endotoxins, virus-like particles, retroviral activity, fungi, yeasts, bacteria, and testing for immunogenicity or tumorigenicity). Overall, FPC transplantation programs as described herein enable optimal efficiency at an industrial level of innovative therapeutic product manufacturing and translation, as well as sustainable and on-demand availability of therapies meeting clinical unmet needs (Laurent et al. 2020b).

Hyaluronan-Based Hydrogels for Cell Delivery of Banked FPCs

Hyaluronan-based vehicles have been investigated for formulation and delivery of equine and human FPC therapeutic cell types (Grognuz et al. 2016; Laurent et al. 2020a). Such formulations present great interest for non-surgical injection delivery of biological therapies to injured tendons, muscles, or other soft tissues (Tezel and Fredrickson 2008). Marketed or authorised commercial injectable products (e.g. Ostenil®, Synovial®, Hyalgan®) were compared in the context of extemporaneous suspension of living FPCs for clinical delivery, allowing for optimal survival of therapeutic materials, as well as acceptable and stable rheological parameters. It was shown that refrigeration storage may be used in case of delays before transplantation (i.e. several days), in view of slowing cell metabolism and significantly prolonging survival. The clear influence of gel viscosity on cell survival rates was outlined, as well as on homogeneity of the preparations. Furthermore, results indicated that small product volumes (i.e. 500 µl) containing several million cells could be extruded through standard-sized needles (e.g. 22-gauge) and stored, while preserving structural integrity, high relative viable cell proportions, metabolic activity, adhesion, migration, and proliferation of therapeutic FPCs. In the specific case of human tendon FPC delivery, it was outlined that Ostenil® Tendon HA (TRB Chemedica SA, Switzerland) presented optimal characteristics for on-demand biological therapeutic combination product development (Grognuz et al. 2016).

Nanoscale Hyaluronan-Based Biological Combination Products for Optimised Regenerative Effects

Both hyaluronan and banked FPCs benefit from extensive historical hindsight and industrial manufacturing applicability evidence, which places such materials in key positions to illustrate modern paradigm shifts towards biological therapeutics development and use. Indeed, emerging evidence lead increasing numbers of researchers and clinicians to investigate alternative treatment options to those proposed by classical pharmacotherapeutic guidelines, focusing on biologically sourced products. Considering the proposed combinations (i.e. FPC-loaded hyaluronan hydrogels), further developments shall shed some light on the optimal processing conditions and target parameters for tailoring and adaptation of raw materials. To this end, a schematic summary of such combination product lifecycles is presented in Figure 8.5. Indeed, for maximised therapeutic benefits to be obtained, hydrogel functionalisation and tuning may necessitate the presence, inherent or acquired, of nanoscale biological particles or aggregates, optimising the delivery or preservation of key therapeutic components. Similarly, standardised cellular materials may be further processed before inclusion in final formulations, with the derivation of nanoscale cellular compartments or components, such as exosomes, microvesicles, or soluble fractions of lysates and conditioned medium. While unfractionated cellular materials may exceed the nanometric scale, native hyaluronan from biological sources has been characterised by means of average root-mean-square radius determination to generally be under 100 nm for polymers around 10^6 Da. Therefore, pragmatic exploitation of high therapeutic value biological raw materials (e.g. hyaluronan and banked primary FPC sources), coupled to sound product development and manufacturing, shall further enable tangible contributions in the wide field of cell therapies and regenerative medicine.

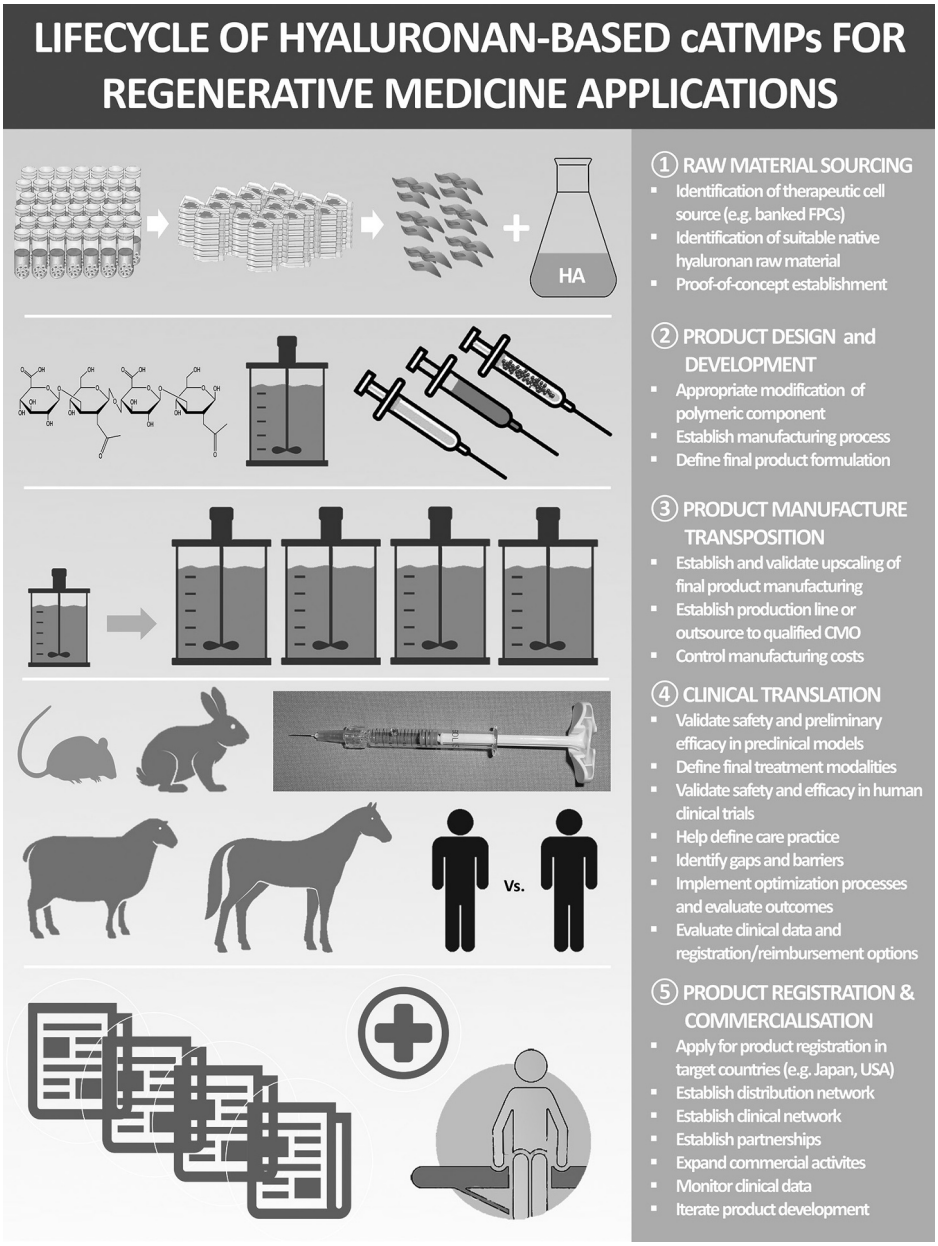

FIGURE 8.5 Overview of developmental activities and steps for creating, registering, and commercialising biological combination products based on hyaluronan and therapeutic cells. The sourcing phase should allow for the identification of optimal therapeutic cell types (e.g. FPCs), polymer structure and properties, and chemistry involved in the formulation. The design and development phase then leads to the formal establishment of specifications, processes, and parameters to obtain working prototypes, or at least a minimal viable product (MVP). Upscaling of manufacturing must then be validated, and production may be outsourced to appropriate and qualified contract manufacturing organisations (CMO). Clinical translation of the product is achieved after appropriate evidence and clinical data has been gathered successfully. The final stage of product registration and commercialisation then marks the beginning of the product-market lifecycle, which must be iteratively optimised and ameliorated, to ensure maximal numbers of patients may benefit from the therapeutic benefits yielded by the product.

Conclusion and Perspectives

The various considerations relative to product development in regenerative medicine, using highly versatile and safe biological raw materials as presented herein, enable us to conclude on the high interest for further industrial development and clinical translation efforts. Hyaluronan-based hydrogels constitute excellent versatile working bases for sound design, development, and manufacturing of innovative cATMPs, related TEPs, and medical devices. Indeed, key physico-chemical properties and parameters of hyaluronan hydrogels may be tailored at will for the optimal support or conjoined effect provision within cATMPs containing standardised cellular substrates or derivatives thereof.

To this end, primary FPCs may be isolated, after a single organ donation, from tissues such as skin, cartilage, tendon, bone, muscle, intervertebral disc, or lung, using enzymatic or mechanical methods for rapid, safe, sustainable, and efficient Parental Cell Bank generation. Subsequent robust and multi-tiered GMP cell banking workflows may be scalably transposed to industrialised processing, providing starting materials for decades of research and clinical applications, potentially addressing the needs of millions of patients. Generation of large stocks of such allogeneic cellular raw materials allows for drastic minimisation of product clinical availability delays.

Furthermore, pragmatic optimisation of combination product developmental workflows enables optimal adequation with modern regulatory and economic constraints relative to cellular therapy products, cell-based products, or cell-based cell-free preparations. The overarching benefits of exploiting standardised substrates such as hyaluronan and banked primary FPCs consist in the demonstrable industrial and clinical experience with such materials, wherein the scalable manufacturing of both components provides a key competitive advantage. Therefore, the critical product design considerations covered in this chapter, directly impacting the potential of proposed formulations in terms of clinical translation and effectiveness, shall contribute to the further development of high clinical value products and therapies for optimised regenerative stimulation and management of wide arrays of cutaneous and musculoskeletal affections globally.

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