

Soft tissue application of biocomposites

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4.1 The multiphase composition of natural tissues: Inspiration from living soft tissue composites

The organs in a human body work in an orchestrated manner to accomplish their responsibilities. Understanding how tissues interact to intrinsically coordinate repair/regenerate or to undergo degeneration can hopefully give important insights to develop engineered composites to support regenerative medicine and tissue engineering approaches to either stop degenerative processes or to guide fully tissue/organ regeneration.

From a material scientist's viewpoint, soft tissues can be envisioned as composite multiphase structures in which distinct phases are dispersed in the aqueous continuous phase of the tissue-specific extracellular matrices, inducing the multifaceted peculiarity of each tissue. A composite is defined (Work et al., 2004) as a 'multicomponent material comprising multiple different (nongaseous) phase domains in which at least one type of phase domain is a continuous phase', extracellular matrix (ECM) itself can be considered a composite. The water swollen macromolecular three-dimensional structure of ECM that highly resembles a hydrogel typically represents the continuous phase. In the case of soft tissues, the dispersed phase could be represented by particles and fibrous structures of either organic or inorganic origin. Thanks to the multiphase structure, ECM controls the diverse physiological functions and the different component functionality within the microenvironment (Huang and Li, 2011; Lin et al., 2016). In addition, multilayered composites are also observed in nature, in tissues such as bone and blood vessel wall, where stiff fibres are embedded in a compliant matrix to form a ply: Multiple plies are stacked in a concentric manner to tune mechanical properties and enhance toughness. There are ubiquitous composite materials in nature at different scale levels, such as fibres immersed in a soft water swollen matrix, amyloid fibrils, stiff, or elastic collagen fibres in the proteoglycans matrix for cartilage (Bas et al., 2017) or for heart valves, skin, intervertebral disc or, at a different scale, microtubules within the cell (Zhao et al., 2016).

Nature is a great source of new ideas and solutions for material scientists to engineer soft tissue features and to develop advanced materials by exploiting strategies already optimised through evolution. The boundary between the two concepts, respectively, referred to as 'biomimicry' and 'bioinspiration', is sometimes blurry, as the base of both definitions is the idea of an imitation of nature (Fig. 4.1). If for general technological application it is possible to agree with Rajeshwar (2012), according Page 1 to

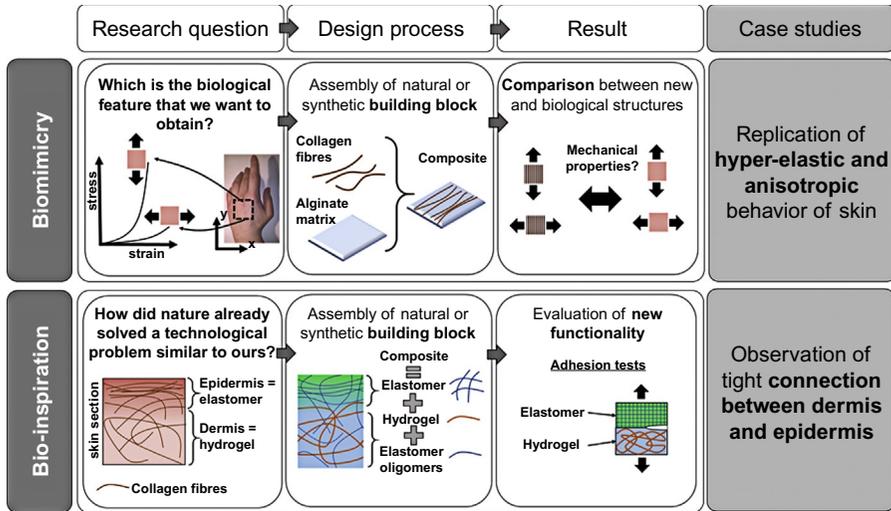


Fig. 4.1 Comparison between biomimicry and bioinspired design process. In the upper part, biomimicry process is described; whereas bioinspiration process is described in the lower one. Examples regarding skin biomimicry and bioinspiration are, respectively, given.

whom bioinspiration is conceptually a step ahead of biomimicry, in the biomaterial field biomimicry is usually a necessity due to the biocompatibility requirements of medical devices. Moreover, the replication of biological processes can be particularly complex, and therefore, in this context, it is not worth creating an intellectual hierarchy. All this considered, when referring to biomimicry, the stress will be on the feature of the biological properties that was intended to be achieved by the man-made structure, whereas bioinspiration will be focused on solving engineering problems by observing the tactics that nature has found, through the ‘trial-and-failure’ approach of evolution, which researchers can successfully apply to develop novel structures.

4.1.1 Soft tissues as structural composites

Physiological components for mechanical stability rely on fibrous macromolecules that cooperatively modulate mechanical stimuli of the cells, influence their shape variation on activation and phenotype expression (Akhmanova et al., 2015). Collagens, together with elastins, fibronectin, and laminins represent the fibrillar structure of ECM (Theocharis et al., 2016). Fibres bring ECM orientation and cell alignment in ordered geometries and soft tissue anisotropy.

Improved techniques have paved the way for the discovery of mechanotransduction – a term that describes the ability of cells to transduce physical stimuli into intracellular biochemical cascades (Discher, 2005; Iqbal and Zaidi, 2005; Ingber, 2006). Major biological processes are governed by this mechanism that range from tissue development (Wozniak and Chen, 2009; Mammoto and Ingber, 2010; Kolahi and Mofrad, 2010; Farge, 2011; Kuo, 2013), regeneration (D’Angelo et al., 2011; Evans et al., 2013; Pioletti, 2013; Qin and Hu, 2014; Duscher et al., 2014), and even disease (Ingber,

2003; Jaalouk and Lammerding, 2009; Papachroni et al., 2009; Seong et al., 2013; Takahashi et al., 2013). Different cellular components have been linked to primary mediation of the mechanotransduction phenomena, including cytoskeleton, adhesion complexes, and ion channels (Clark et al., 1987, 1990; Curtis and Wilkinson, 1997).

4.1.2 Soft tissues as composite hydrogels

In soft tissues, a variety of substances diffuse from very specific microparticles, the cells, and migrate through diffusive mechanisms within the water swollen matrix, the ECM (Kihara et al., 2013; Schiller and Huster, 2012), resulting in a heterogeneous environment where both the matrix and dispersed phase contribute to regulate the process. Specifically, proteoglycans and hyaluronans (Theocharis et al., 2016) induce the hydrogel nature of native ECM from soft tissues, therefore regulating the water content. This affects the diffusion of growth factors, cytokines, enzymes, and any substance by determining water content, the matrix mesh size and also the chemical moieties present on their backbone. Their negative charge and the charge density, as well as the type and extent of functional groups are often targeted features to be mimicked. The fibrous dispersed phase still contributes by steric effect to the process.

Owing to their similarity to ECM, natural hydrogels became the materials of choice for different biomedical purposes, as they provide three-dimensionality that cells experience in vivo, high water content, adjustable mechanical properties, and assisted diffusion to gases and nutrients (Tibbitt and Anseth, 2009; Geckil et al., 2010; Pacheco et al., 2015a). In a more comprehensive approach, ECM can be mimicked by composite hydrogels (Zhu and Marchant, 2011), where the hydrogel matrix is fundamental to regulate diffusion and it induces the mechanical cell stimulation by transmitting the external forces, whereas the fibrous structure is involved in the regulation of diffusive processes.

4.1.3 Soft tissues as multifunctional composites

The advantageous properties of natural composites, compared with single-phase materials, may not fall solely into the biomechanical issues following the reinforcement effect of the dispersed phase. In addition to structural integrity, in the composite nature of ECM lays its biological activity, mechanotransduction, and diffusion mechanisms of signalling molecules, metabolites, and catabolites. This multiphase structure is the key to regulate the fate of cell recruitment and proliferation, as well as degradation and remodelling of the tissue itself. ECM structure (Theocharis et al., 2016) is strictly connected to its functionality such as biochemical signalling (Zhu and Marchant, 2011).

In addition, the typical alignment of the neuronal network in the brain, myofibrils in both heart and skeletal muscles, as well as collagen fibres in the skin, ligament, and tendon (Kim et al., 2013) indicates that the possibility to control topography pattern and orientation holds great potential for different tissue engineering purposes (Kim et al., 2013). Topographical features have an impact on stem cell phenotype, namely, the pattern of topography, size of these features as well as chemical composition (Hodde et al., 2016; Janson and Putnam, 2015).

4.1.4 Biophysical cues of soft tissue composites

Communication among the different organs/systems is of utmost importance for a proper human function. Not so distant, these communications were believed to occur mainly from cell-to-cell through biochemical signalling. Hence, mechanical forces were less common tools among medical treatments. Signals are related to biochemical and biophysical cues as electric, magnetic, and piezoelectric. Biophysical cues exhibit advantages in comparison with biochemical signals, including longer duration (i.e. they are not removed during medium change), ability to be better characterised, as well as higher reproducibility (Villa-Diaz et al., 2013; Ding et al., 2017).

The biological electric field of the human body is a recognised natural phenomenon, which electrical potentials span around 10–60 mV in different parts of the body (Foulds and Barker, 1983). Electrical signals have tremendous impact on cell behaviour, as it promotes permeation of ions across the cellular membrane that changes its potential and alters transduction pathways (Mattioli-Belmonte et al., 2003). Bioelectricity is inherent to wound healing; its presence guides cell migration to the wound edge, whereas its absence inhibits tissue repair/regeneration (McCaig et al., 2005). As an example, cardiomyocytes require dynamic microenvironment that address not only biochemical and biomechanical cues, but also the appropriate electrical stimulation (Nakayama et al., 2014; Amezcuca et al., 2016).

Piezoelectricity – mechanical pressure that is converted into electric charge, or vice versa (Bassett, 1967) – in turn, is a phenomenon that has also been associated to many proteins that make part of the ECM composition such as collagen (Fukada and Yasuda, 1964), keratin (Telega and Wojnar, 2002), elastin (Fukada and Hara, 1969), myosin, and actin (Ueda and Fakada, 1971). The piezoelectric property of collagen has been investigated in composite biological systems such as bone, tendon, and skin, which mostly consist of collagen fibres. The effect is also perceived in DNA, hair owing to keratin, ligament, muscle, blood vessel wall, and intestines, among others. Despite its well-documented impact over tissue remodelling, and possibly DNA copying and glucose transformations, piezoelectricity is currently an overlooked property that is not properly exploited in tissue engineering.

By recognising the pathway in which cells convert physical stimuli into biochemical activity, that is, mechanotransduction, more advanced and biomimetic composites have been developed.

4.2 Engineered biocomposites for soft tissue application

As nature produces soft composites with remarkable mechanical, sensing, and actuation properties, the most conservative and straightforward path to successfully guide tissue regeneration would rely on the creation of biomimicked hydrogels starting from decellularised tissues (Saldin et al., 2017). In this sense, tissues from different organs (heart, brain, kidney, and lungs) are solubilised through enzyme activity and neutralised, and its residues can be subsequently crosslinked into ECM hydrogel

composites. Such composites exhibit enhanced similarity to human tissues, as they retain all the different elastin, collagen, and glycosaminoglycan components of the original tissue (Saldin et al., 2017). However, some peculiar properties such as structural features, spatial organisation, and composition are in part altered during the process (Fu et al., 2016).

Engineered composites are a promising alternative for tissue regeneration purposes, as they mimic or take inspiration from the composite nature of soft tissues, and have sprouted innovative techniques of material design and functionalisation. For this, current experience on implantable biomaterials (Peppas et al., 2006; Williams, 1996) and their specific functionalisation techniques (Garner and Park, 2014; Li et al., 2016; Munarin et al., 2012) has been gathered and consequently the limitations of nature-retrieved materials being evaded.

Considering the vast and evolving scenario of composites for soft tissue application, representative examples of applications will be focused on composite materials where hydrogels are the continuous phase. The role of the dispersed phase represented by either fibres or particles will be extensively discussed in terms of final properties of the composite hydrogels, multifunctional nature, and ultimately over cell behaviour (Fig. 4.2).

4.2.1 Biomimetic and bioinspired structural biocomposites

The anisotropic properties of soft tissues can be designed using fibre-reinforced composites that mimic the layout and orientation of natural fibres, and therefore matching those of natural tissues, namely, highly oriented soft tissues – muscles, myocardium, blood vessels, adipose tissue, and cartilage. The composite design can move as far as producing injectable fibrous (Poveda-Reyes et al., 2015) or particle-reinforced (Munarin et al., 2014, 2015) gels. In this regard, hydrogel reinforcement aims to mimic the interactions between dispersed-phase matrix of soft tissues, without compromising injectability and its chemical characteristics.

Wounds from deep burns generally involve the whole skin thickness, which compromises skin structure, appendages, and immunitary system and therefore impairing skin regeneration. One of the key parameters to successfully guide wound healing is mimicking the double-layer nature of human skin: a softer layer that matches the properties of the underlying tissues and a thicker external layer that prevents water evaporation. With this in mind, the bilayered skin structure was designed using a chitosan hydrogel with different densities bounded through the exposure to ammonia vapours. The resultant biocomposite presented a better performance in terms of neovascularisation in the mid-term and mechanical properties in the long-term, with respect to the controls (Boucard et al., 2007).

In tissue engineering, physical properties such as permeability and hydrophilicity are often neglected or are not targeted as the most important to be mimicked. Nevertheless, these features play an important role in biointegration. Electrospun composite matrices made of chitosan/cellulose blends exhibited both contact angle and oxygen permeability in the same order to those of human skin, thanks to the addition of medical-grade nanodiamond particles, which have also promoted superior matrix stability and made suitability for wound dressing (Mahdavi et al., 2016).

	Application	Biomimicked feature	Engineered solution
Single function	Wound healing of deep burns	Bi-layered mammalian skin (dermis + epidermis)	Double layered collagen film → different polymer density
		Permeability and hydrophilicity properties of the skin	Electrospun chitosan/cellulose matrix with dispersed nanodiamond
	Large vessel graft	Anisotropy and nonlinearity of vessel wall	Soft multiply composite: collagen fibres in alginate matrix
	Wearable electronics	Elasticity and toughness	Chiral honeycomb (+ conductive circuit) embedded in deformable matrix
		Self-healing properties	Self-healing polymer + conductive microparticles (nikel, carbon nanotube)
	Implantable electronics (sensing)	Mechanical properties of the targeted tissue: functionality not biomimicked by biological tissue	<ul style="list-style-type: none"> • Silicon • Collagen • Polyacrylamide + alginate • Silk fibroin (bioresorbable) } Flexible electronic arrays
Drug release	Fast diffusion of active molecules in vivo → mechanism to be opposed, not mimicked	Drug-loaded gel or polymer particle embedded in a gel matrix	
Multifunctional	Heart tissue regeneration	Tissue deformability	Carbon nanotubes embedded in methacrylated gelatin gel: nanotubes can be either coated with albumin or functionalized
	Nerve regeneration	Electrical conductivity	
	Muscle tissue regeneration	Tissue deformability	Polypyrrole dispersed on sponge-like gellan gum hydrogels

Fig. 4.2 Classification of the applications of soft biomaterial composites: at macrocategory level it depicts the difference between single function application and multifunctional materials. The application is related to the biomimicked feature and the correspondent engineering solution.

To achieve mechanical compatibility, the elastic properties should be mimicked considering the nonlinear and nonisotropic behaviour of soft tissues. The vessel wall, for example, is constituted by layers of collagenous fibres oriented with different angles in respect to the principal axis. [Sharabi et al. \(2015\)](#) produced multilayered laminates made of collagen fibres embedded in an alginate matrix. Aiming to obtain hyperplastic and anisotropic behaviour similar to human aorta, the bioplies were stacked in different sequences. In the future, such composites could be used to produce large vessel grafts with mechanical properties close to the vessel itself to minimise stress concentration at the interface between a graft and healthy tissue, which is on the basis of graft failure ([Sharabi et al., 2015](#)). Interestingly, to obtain the desired mechanical properties, traditional composites designs were adopted, where plies made of the synthetic components were stacked one on the top of the other with a different orientation with respect to a reference

axis, thus reporting an engineering-inspired approach to a biomimetic problem. To finely tune the properties of stretchable composites to match those of biological materials, bidimensional cellular structures were embedded with a chiral geometry in a deformable matrix. The honeycomb geometry enabled to tune the nonlinear and anisotropic properties to match different soft tissues, and therefore widening the possibility of applications, ranging from wearable electronics to drug delivery (Jang et al., 2015).

Scaffold stiffness acts as a mechanical cue that tends to be more effective than soluble factors (Engler et al., 2006). According to the intended application, the stiffness of the scaffolds can be bioinspired to control cell differentiation towards specific lineages. Specific stiffness ranges have shown predictive outcomes. In this sense, brain-mimicking surfaces (0.1–1 kPa) guided differentiation towards neuronal lineages, muscle-like surfaces (8–10 kPa) hold myogenic potential, whereas stiffer bone-like surfaces (25–40 kPa) guided osteogenesis (Rehfeldt et al., 2007; Vidane et al., 2013) (Fig. 4.3).

The importance of mechanical properties was, indeed, proved in studies that used ECM injectable hydrogels to stimulate brain regeneration, where minimally invasive therapies are required (Saldin et al., 2017). ECM hydrogel composites made of decellularised urinary bladder matrix was proposed for brain damage treatment. The rheological properties played a pivotal role over matrix colonisation. Low-viscosity hydrogel composites were not able to fulfil properly the injury, and thus the cells

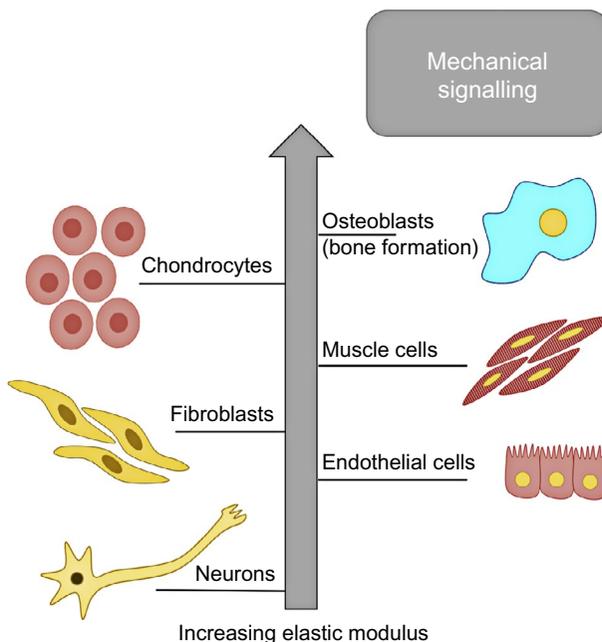


Fig. 4.3 Schematic illustration depicting the influence of ECM stiffness over cell activities, as well as the characteristic *in vivo* ranges according with the tissue ECM.

remained at its borders, whereas more viscous hydrogels composites promoted cell migration and provided a storage modulus of about 460 Pa, which is close to the stiffness of brain tissue (Massensini et al., 2015).

Moving from the use of employing decellularised tissues for biomimetic and bio-inspired gels, the latter offer a larger versatility. In fact, depending on the aim of the study, the reproduction of both composition and structure of tissues is usually not required. Instead, the reproduction of a specific function or microenvironment, as release of growth factors to stimulate cell proliferation and avoid dedifferentiation, are more relevant to promote tissue regeneration and achieve proper functioning. As an example, chondrocyte-loaded composite gels for cartilage tissue engineering consisting in oligo [poly (ethylene glycol) fumarate] embedding gelatine microgels, carrying growth factors specific for chondrocytes, enhanced cellular proliferation, and provided the maintenance of cellular phenotype. The goal was reached, also thanks to the tailored mechanical behaviour and controlled drug release kinetic given by the gel-in-gel composite structure (Holland et al., 2005).

Unlike synthetic structures, animals, and plants possess the unique ability to self-heal (Huynh et al., 2017). In the last decade, this ability has inspired researchers to develop a new generation polymers with 'intrinsic' self-healing, therefore differing from the previous generation that held a reservoir of monomers and catalysts embedded in vesicles coupled to the main matrix (Huynh et al., 2017). These polymers, in fact, exploit the reversible molecular interactions of hydrogen binds, π - π stacking or metal-ligand coordination (Roy et al., 2015) and become suitable for wearable electronics when doped with conductive nickel microparticles (Tee et al., 2012), carbon nanotubes (Guo et al., 2015), or carbon nanowires (Sun et al., 2014). The most advanced technologies show that it is possible to induce self-healing of non-self-healing layers, when these are deposited onto self-healing substrates, therefore resulting in a self-healing multilayer (Huynh and Haick, 2016). Nevertheless, the healing process is very different from that of the physiological one, as (to date) there are no living cells in synthetic composite matrices able to produce new tissues. Yet, it is possible to observe nature and learn lessons on how its nontrivial engineering problems were solved. For instance, in soft tissue it is crucial to connect components with antithetic properties through a strong link without creating weak points at the interface (Stuart, 2016). As previously mentioned, skin can strongly bind a hydrogel-like layer (dermis) to an elastomeric superficial layer (epidermis), resulting in a tough interface that enables its coupling to functional microstructures (sensors and blood vessels). With this in mind, scientists created a tough hydrogel composite through a versatile method that consists in infiltrating physically crosslinked hydrogel networks with monomer molecules. The obtained hydrogel is then positioned on top of an elastomeric layer, properly treated to be more reactive, and the crosslinking between the two structures takes place at its interface. The resultant bilayered structure has an interfacial strength 10 times tougher than mammalian skin and was able to embed working circuits and microchannels (Yuk et al., 2016).

Mammalian tissues are not the only structures scientists can find inspiration. Mussel byssus is a thin, but resistant filament that connect mussels to the substrates and its proved highly resistant to impact character is related to the gradual stiffness

gradient along its length (Qin and Buehler, 2013). Inspired by this nonmineralised composite, scientists have developed polymer gradient materials without interface or discrete layers of mechanical properties (Claussen et al., 2012), that actually proved to be a critical factor in damage resistance (Qin and Buehler, 2013).

4.2.2 Biocomposites to control molecular diffusion

Hydrogels are often incapable of mimicking the proper cell niche to promote accurate *in vitro* tissue development. The recapitulation of the biochemical cocktail of tissues microenvironment is crucial to regulate and guide cell behaviour (attachment, proliferation and differentiation) towards complete regeneration (Tibbitt and Anseth, 2009; Geckil et al., 2010; Pacheco et al., 2015a). These biochemical cues can be either provided directly or released in a controlled manner after their loading. Controlled release offers many advantages over traditional, including reduced amount of administered drug with optimised activity, decreased side effects, and release in a spatiotemporal fashion (Varde and Pack, 2004; Tiwari et al., 2012; Laura et al., 2016). Yet, on implantation the micro/nanoparticulate systems are submitted to external mechanical deformation, uncontrollable dislocations, and premature metabolisation prior to substance release that have a direct impact on tissue regeneration (Lemperle et al., 2004; Pacheco et al., 2015b). To tackle this problem, micro/nanoparticulate systems have been physically and/or chemically combined with hydrogels, therefore offering versatile composites able to mimic the structural and biochemical cell niche, and therefore positively influencing tissue regeneration (Jia and Kiick, 2009).

4.2.2.1 Biocomposites to guide tissue regeneration

Hyaluronan (HA)/methyl cellulose (MC) hydrogels with dispersed poly(lactide-co-glycolide) (PLGA) nanoparticles were proposed as a combinatory strategy to protect and repair spinal cord injury (Baumann et al., 2009). Two neuroprotective molecules, namely, NBQX and fibroblast growth factor (FGF-2) were impregnated on the HA/MC hydrogels as their fast release is desirable on the first stage of the injury, whereas dbcAMP, epidermal growth factor, α -chymotrypsin, and IgG were incorporated on PLGA nanoparticles, owing to its neuroregenerative role. As expected, NBQX and FGF-2 were promptly released after 1 and 4 days, respectively, whereas the four neuroregenerative molecules exhibited a sustained release over 28 days. The composites displayed significantly decreased *in vitro* degradation in respect to the hydrogel alone, with high residual particle load (Baumann et al., 2009). In addition, after dispersing the PLGA nanoparticles, the composite presented a twofold increase on storage modulus in respect to HA/MC hydrogels, possibly owing to hydrophobic interactions between MC and PLGA, suggesting crosslinking formation (Baumann et al., 2010). On implantation, HA/MC hydrogels did not elicit any inflammatory response, whereas HA/MC composites displayed a mild reaction. Nevertheless, both HA/MC hydrogels and composites did not trigger astrogliosis, associated to inhibition of spinal cord regeneration (Lemons et al., 1999), or hampered motor function on either uninjured or injured animals (Baumann et al., 2010). In a follow-up study,

cyclosporin A was incorporated in the previously described composite (i.e. HA/CM hydrogel with dispersed PLGA particles), aiming to improve the survival of endogenous neural stem/progenitor stem cells after brain implantation for the treatment of stroke (Caicco et al., 2013). The release of cyclosporin A from the HA/MC composite exhibited significantly reduced burst effect and prolonged release for over 28 days, in accordance with the previous studies (Baumann et al., 2009, 2010; Stanwick et al., 2012; Caicco et al., 2013). HA/MC containing PLGA loaded with cyclosporin A was further injected into the cortex of mice to assess in vivo brain tissue penetration of the drug. Cyclosporin A was identified up to 3000 μm in depth (about 60–65% of the total dorsal–ventral dimension of mouse brain) and its concentration was constant up to 24 days after implantation (Caicco et al., 2013). Bora et al. proposed a similar system as composite drug delivery system for glaucoma treatment (Bora et al., 2016). After dispersing 5-fluoracil-loaded PLGA microspheres in different concentrations of methacrylated HA hydrogels, it was observed that favourable injectability was achieved when using 0.5% of HA. Unlike the previously described works, 0.5% HA hydrogels did not impact the drug release rate, which was similar when using PLGA particles alone. Nevertheless, for higher concentrations of HA, delayed drug release was experienced (Bora et al., 2016).

4.2.2.2 *Biocomposites for cancer treatment*

Particulate systems have been also exploited to tackle different types of cancers owing to its ability to prolong drug exposure within the tumour mass, while presenting less toxicity and improved antitumor activity (Elstad and Fowers, 2009; Cao et al., 2012). Injectable graphene oxide hydrogels were designed by supramolecular assembly with polyvinyl alcohol and used as a dispersion platform of camptothecin-loaded β -cyclodextrin nanoparticles for the treatment of cancer (Ye and Hu, 2016). The β -cyclodextrin nanoparticles possessed pH sensitivity that is of utmost importance for cancer treatment, as tumour tissue microenvironment is known for its acidity (Kato et al., 2013), and therefore were able to specifically control drug release. At an acidic pH, a higher release of camptothecin was experienced, which was more pronounced as more acid was the released in the environment. In agreement with Shoichet and co-workers' studies (Baumann et al., 2010; Stanwick et al., 2012), the embedding of β -cyclodextrin nanoparticles affect the final viscoelastic properties of the graphene oxide hydrogels, and therefore stiffer gels were obtained with the increase of nanoparticle concentration, which also confer higher physical stability over a wide range of frequencies (Ye and Hu, 2016).

4.2.3 *Multifunctional biocomposites*

Multifunctional biocomposites are structures composed by hydrogels with dispersed advanced particles and/or materials that undergo changes in conformation in response to external stimuli (Biondi et al., 2015; Mehrali et al., 2016; Gaharwar et al., 2014). Among the different external stimuli, pH (Ye and Hu, 2016; Wang et al., 2010), thermal variations (Wang et al., 2004; Zhang et al., 2011; Yassine et al., 2016), electricity

(Xu et al., 2010; Berti et al., 2017; Cui et al., 2014; Guarino et al., 2013; Balint et al., 2014) and magnetic fields (Li et al., 2013; Tampieri et al., 2014; Tóth et al., 2015; Häring et al., 2015) are the most proposed within tissue engineering and regenerative medicine. Coupling biophysical cues to an already existing scaffold has gathering great interest owing to its proved capability of directing stem cell behaviour by either maintaining their stemness or managing differentiation into specific lineages (Ding et al., 2017) (Fig. 4.4). The nature of the displayed functional properties can be tailored accordingly with the chosen particle or material that is dispersed within the hydrogel networks, therefore resulting in multifunctional biocomposites.

4.2.3.1 Electroactive soft biocomposites

Carbon nanotubes (CNTs) are ‘one-dimensional’ carbon structures made of trundled graphene sheets that exhibit mechanical resilience combined with lightness, elasticity, and nanotopographic features (Tozzi et al., 2016; Marchesan et al., 2016). These features together with optical, thermal, and electronic properties make them promising materials for regenerative medicine – as adjuvants to scaffolds – and pharmaceutical – as theranostic tools (i.e. therapeutic effect combined with diagnostics) (Marchesan et al., 2015, 2016). Owing to its elasticity, CNTs can be dispersed in hydrogels without hampering their initial properties (Tonelli et al., 2012). Their intrinsic conductivity has shown to positively influence cell adhesion and consequently regeneration of cardiac (Shin et al., 2013; Mooney et al., 2012) and nerve tissues (Tosun and McFetridge, 2010; Lee et al., 2014; Liu et al., 2016). Hydrogel composites composed of methacrylated gelatin (GeMa) and CNTs with seeded cardiomyocytes were fabricated into ultrathin cardiac tissue patches for cardiac tissue regeneration (Shin et al., 2013). The CNTs of the hydrogel composite did not affect porosity and pore size distribution of GeMa hydrogels. Yet, the hydrogel composite exhibited fibrous networks resembling the Purkinje fibre networks, present on the surface of heart muscle tissue, which were not observed on GeMa hydrogels. After homogeneously dispersing the CNTs, the hydrogel composite presented a threefold

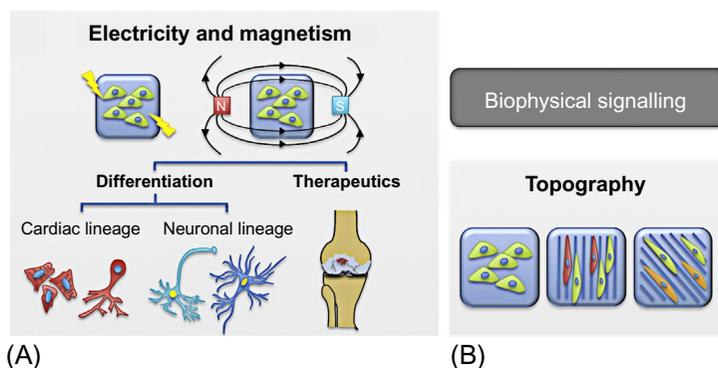


Fig. 4.4 Illustration depicting the impact of different biochemical cues over cell activities, including: (A) electric and magnetic stimulation, and (B) topography.

increase in elastic modulus in respect to GeMa hydrogels that highly resembled the elastic properties of adult rat ventricular myocardium. Apart from its advanced electrophysiological functions, hydrogel composites exhibited improved cardiomyocytes attachment, viability, alignment, and elongation than GeMa hydrogels. Nevertheless, after 6 days a higher number of cells was observed on GeMa hydrogels than hydrogel composites. On electrical stimulation, cardiomyocytes seeded on hydrogel composite showed more stable spontaneous beating and beating rate than those cultured on GeMa hydrogels. Finally, the cardiomyocytes seeded on GeMa hydrogels showed rupture of tissue integrity due to electrical stimulation, which was not observed on hydrogel composites that promoted higher mechanical integrity/resistance of the cells (Shin et al., 2013). CNTs can be further functionalised with proteins (Nagaraju et al., 2015). Albumin-coated CNTs collagen hydrogel composites were proposed for nerve regeneration (Tosun and McFetridge, 2010). The dispersion of albumin-coated CNTs on collagen hydrogel composites was more homogeneous after the addition of sodium cholate than in its absence, which led to higher hydrogel fibre diameter. Improved conductivity and compression modulus were attained when the hydrogel composite contained albumin-coated CNTs. Although PC12 cell proliferation was enhanced for both types of hydrogel composites at initial time points, at prolonged culture times their effect was minimised in relation to collagen hydrogels (Tosun and McFetridge, 2010). A similar platform was proposed by Lee et al., though before dispersion on collagen hydrogels, the CNTs amino-functionalised (Lee et al., 2014). Hydrogel composites displayed decreased fibre diameter in comparison with collagen hydrogels, which is in dissent to Tosun and co-workers' study (Tosun and McFetridge, 2010), possibly owing to the enriched solubility of the amino-functionalised CNTs. Yet, no significant differences on mechanical properties were observed between the developed hydrogel composites and collagen hydrogels. Mesenchymal stem cells proliferation was favourably enhanced in hydrogel composites up to a CNTs concentration of 1 wt%, which also exhibited a positive effect over cell elongation and differentiation towards neuronal lineages that was translated by the upregulation of neural markers (Lee et al., 2014). However, the cell behaviour in the presence of electrical field was not reported in both studies.

Dispersing polyheterocycles, as polypyrrole (Berti et al., 2017), or grapheme oxide (Tungkavet et al., 2015) within hydrogels can also render soft composites electroactive. In this regard, Berti and co-workers have studied cellular behaviour on electroactive hydrogel composites made of polypyrrole dispersed on sponge-like gellan gum hydrogels (Berti et al., 2017). Hydrogel composites displayed enhanced conductivity and hydrophobicity, as well as decreased pore size, porosity, and stiffness in comparison with gellan gum hydrogels. In agreement to the previously reported studies, both fibroblasts and C2C12 myoblast cells presented higher elongation on hydrogel composites rather than gellan gum hydrogels (Berti et al., 2017).

Electrical stimulation exhibits positive influence over cell behaviour, mainly those of electroactive tissues as muscle and brain. In general, cardiomyocytes present a superior behaviour similar to native cardiac tissue, including cell elongation, organisation, alignment, metabolic activity, and autonomous beating (Shin et al., 2013; Eng et al., 2016). Electrical fields have also displayed influence on neuron migration, rate, and

direction of neurite outgrowth, as well as differentiation towards neuronal lineages (Yao et al., 2011; Park et al., 2012) (Fig. 4.4A).

4.2.3.2 *Magnetic soft biocomposites*

Magnetism is a promising tool that has allowed researchers to remote control cell activities noninvasively by applying an external magnetic field (Ito and Kamihira, 2011). Magnetic biocomposites can be advanced through the combination of hydrogels with magnetic micro- and/or nanoparticles, for example, magnetic iron oxides. Within the biomedical context, magnetic composites have been proposed as supports for tissue regeneration (Ito and Kamihira, 2011), drug and gene targeting and delivery (El-Sherbiny et al., 2017), and as magnetic resonance imaging contrast agents (Estelrich et al., 2015) (Fig. 4.4A). Hydrogel composites made of hyaluronate hydrogels with dispersed ferromagnetic nanoparticles was proposed by Tóth et al. as injectable regeneration supports for osteoarthritis therapy (Tóth et al., 2015). On coating the magnetic nanoparticles with chondroitin sulphate, a particle with a mean size of 150 nm was obtained, which after dispersion on hyaluronate hydrogels did not affect its initial features (Tóth et al., 2015). Nevertheless, its capabilities to act as articular scaffolds are yet to be reported. In another embodiment, magnetic alginate hydrogel composites were produced following the dispersion of ferromagnetic nanoparticles, the crosslinking of which took place within the microfluidic device (Hu et al., 2014).

4.2.3.3 *Micro and nanopatterned soft biocomposites*

Scaffold micro- and nanotopography has shown a tremendous impact over cellular morphology and behaviour (Ding et al., 2017; Kim et al., 2013) (Fig. 4.4B). Apart from cell source, different surface features have impact over stem cell phenotype, namely, the pattern of topography, size of these features as well as the chemical composition (Hodde et al., 2016; Janson and Putnam, 2015). Controlled topography has shown enhanced adhesion and spreading of distinct cell types onto ‘nanoislands’ 10–20 nm in height (Dalby et al., 2014), as well as improved Schwann cells’ elongation on PCL nanofibres embedded within fibrin hydrogel composites (Hodde et al., 2016). The sole use of nanopits-patterned surfaces, without any molecular additive, induces mesenchymal stem cells differentiation towards osteogenic (Dalby et al., 2007), mesodermal lineages (Kingham et al., 2013). Embryonic stem cells have undergone differentiation into neuronal lineage after being cultured on nanoscale ridge/groove pattern without addition of differentiation agents (Lee et al., 2010). Likewise, nanoscale groove/pillar patterned surfaces promoted differentiation of neural stem cells into both neurons and astrocytes (Yang et al., 2013). In addition, nanotopographical features have also preserved stemness of cells for long-time periods (McMurray et al., 2011; Tsimbouri et al., 2012). The possibility to control topography pattern and orientation holds great potential for different tissue engineering purposes, as this technique is able to reproduce the alignment of the neuronal network in the brain, myofibrils in both heart and skeletal muscles, as well as collagen fibres in the skin, ligament, and tendon (Kim et al., 2013).

4.2.4 Composites to monitor biological signals

Bioelectronics is an emerging research field that relies on the crosstalk between materials science, electronics, and biology. Within the medical theatre, bioelectronics can act as diagnostic and/or therapeutic tools. The possibility to integrate bioelectronics with living tissues enables in situ real-time processing of biological signals, which can be applied as artificial electronic skin, muscles (Zang et al., 2013; Guo et al., 2014), and cochlear (Wallace et al., 2009), smart soft contact lenses capable of monitoring glucose gradients (Ferri et al., 2011), neuron-to-machine interfaces, remote motorisation of cardiac signals, controlled drug delivery and in different chronic diseases as Parkinson's and diabetes (Gaikwad et al., 2011; Duan et al., 2013; Löffler et al., 2017).

Traditional electronics rely on sensors placed on rigid, brittle, and semiconductive wafers. Their lack of conformability and flexibility hampers their implantation in the human body, and therefore much effort has been made to develop innovative semiconductive substrates and new sensing materials and designs envisioning the development of stretchable, foldable, flexible, adhesive, and biocompatible electronics that can adjust to tissue and body motions (Kim et al., 2011; Wang et al., 2014, 2015; Ge et al., 2016). Enzyme interaction to detect the presence of glucose (Ferri et al., 2011), impedance recording of the cerebrospinal fluid to stimulate neural interfaces (Wallace et al., 2009), and skin-wearable devices to monitor vital heart signals are among the envisioned applications for this emerging research field (Rivnay et al., 2014; Khan et al., 2016). In an attempt to overcome the discrepancy between the rigidity of traditional electronics and the curved and soft surfaces of human tissues, active electronic components have been fashioned as tiny and flexible arrays (Guo et al., 2014; Deng et al., 2014), being mainly produced by printing conductive inks with high accuracy/fidelity (Someya et al., 2016). Nevertheless, these electronics components demand isolation from body fluids to circumvent current outflows to tissues and constraint their degradation and/or corrosion (Lee and Shin, 2007). With this in mind, biocomposites composed of soft plastics that integrate flexible electronic arrays have been extensively proposed as soft bioelectronics. Plastic films allow the reduction in weight and thickness, confer mechanical robustness and flexibility (Editor, 2013), and protect the electronic components. Silicon is the most proposed material that meets most of these requirements; nevertheless, it is of utmost importance to seek for more natural and compatible materials as collagen (Moreno et al., 2015), silk fibroin, and alginate, aiming to improve integration between tissue and bioelectronics. In an attempt to improve the compatibility of synthetic-based electronics, hybrid bioelectronics composed of polyacrylamide and alginate gels were proposed as flexible and stretchable with robust adhesion between these hydrogels and other surfaces (Yuk et al., 2016), and therefore have been proposed as skin micropatches for either drug delivery or to monitor skin metabolites (Dutkiewicz et al., 2017). Instead, Moreno et al. have produced flexible collagen films with gold and magnesium serpentine-like patterns previously deposited through electron beam evaporation (Moreno et al., 2015). This collagen-based flexible films hold transparency within the visible spectrum and mechanical compliance with soft tissues, without visible lamination of the electronic components. In addition, the thickness and

mechanical properties of the films can be tailored by adjusting the concentration of collagen. Silk fibroin is a promising biomaterial for the development of flexible and conformable electronics owing to its ability to control the degradation rate (Zhu et al., 2016). Kim and co-workers developed ultrathin ($<10\mu\text{m}$) bioresorbable silk fibroin films to monitor physiological brain activity (Kim et al., 2009, 2010). These films integrated polyimide-coated gold electrodes array that were bonded to an anisotropic conductive film, which enabled external data acquisition. In this work, the silk fibroin films function as support material of the electronic array that on implantation dissolved in a short-time frame resulting in decreased bending stiffness and improved conformability. After implantation, *in vivo* neural measurements were successfully acquired, the signal of which was improved as the silk fibroin films dissolved with no evidence of immune response up to 4 weeks of grafting (Kim et al., 2010).

4.3 Conclusions: Engineered composites for soft tissues

Overall, the different phases present in soft tissues regulate complex functionality. Soft tissues can be considered according to their specific function: structural composites – where the dispersed phase contribute to the mechanical microenvironment; hydrogel composites – where the water content regulates diffusion mechanisms; and multifunctional biocomposites – thus including the signalling inducing cell fate.

Soft tissue application of composites relies on their similarity to the composite nature of soft tissues, where natural hydrogels represent the continuous phase, and the dispersed phases bring the peculiar characteristics of each tissue. The high and sophisticated specialisation of the natural process can be somehow mimicked. Biomimetic composites may represent a possible approach to answer the challenge of the development of biomedical materials to be applied in substitution or in aid to soft tissue regeneration. An alternative approach is represented by producing hybrid composites by using a natural structure (e.g. a decellularised tissue) in combination with a synthetic material. The most flexible and promising approach could lay in the bioinspired composite materials, where the versatility of the engineered materials could be a tool to capture the complexity of the soft tissues.

Synergistic benefits can be derived from the use of composite materials for soft tissue application that span from the classical applications to new emerging applicative fields challenging the composite material design, such as flexible implantable electronics and skeletal and cardiac muscle regeneration, where the electrical and physical features can be brought by the composite nature of new materials.

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