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Recent Advances in Biomaterials for Tissue Engineering and Controlled Drug Delivery

Christophe Hélary[1] and Martín Federico Desimone[2,*]

C. Hélary

[1]*Sorbonne Universités, UPMC Univ Paris 06, CNRS, Collège de France, Laboratoire de Chimie de la Matière Condensée de Paris, 11 place Marcelin Berthelot, 75005 Paris, France;* [2]*IQUIMEFA-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 Piso 3º, (1113) Ciudad Autónoma de Buenos Aires, Argentina*

M.F. Desimone

Abstract: In this review, recent advances in biomaterials developed to favor tissue repair are presented. The focus is particularly on devices used to promote bone repair, skin wound healing and nerve regeneration. In each case, the specifications for an ideal substitute and the recent advances in the field of these biomaterials are presented. Alternatively, drug delivery systems associated with biomaterials have been developed over the recent decades to stimulate wound healing without any side effects. For this purpose, the overview presents recent advances in medicated dressings for controlled release of antibiotic to prevent infections, growth factors to promote tissue regeneration and gene delivery to modulate cell phenotype.

Keywords: Antibiotics, biomaterials, gene delivery, growth factors, tissue engineering.

1. INTRODUCTION

Tissue engineering is defined as "the application of principles and methods of life sciences and engineering towards the development of biological substitutes that restore, maintain or improve tissue function" [1]. In other words, tissue engineering is the marriage of materials chemistry with cell biology. Unlike prostheses, tissue engineered devices do not have to mimic the physical properties of the damaged tissue, but must provide the appropriate conditions for tissue repair [2]. The physical properties are weaker than those of prostheses but they acquire properties closed to the native tissue over the time (thanks to remodeling by cells). Two strategies are used to fabricate tissue engineered devices. The first strategy aims at generating materials with an open pore structure which permits cell infiltration. The second relies on the development "living implants". This method requires a long period of cell culture within the biomaterial [2]. Overall, biomaterials have to provide chemical and physical cues to host cells to permit tissue regeneration [3, 4].

To promote tissue repair, the addition of biomolecules within the biomaterial is desirable. In pharmaceutical terms, the entire dose of a drug can either be rapidly released or delivered with a sustained and controlled manner. When the biomolecule is not retained within the biomaterials, it can diffuse into blood and the expected effect is shortened. For these reasons, drug delivery systems associated with

*Address correspondence to this author at the IQUIMEFA-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 Piso 3º, (1113) Ciudad Autónoma de Buenos Aires, Argentina; Tel:/Fax: +54-11-49648254; E-mail: desimone@ffyb.uba.ar; and christophe.helary@upmc.fr

In memoriam of Prof. Dr. Luis E. Diaz.

biomaterials have been developed over the last decades to stimulate wound healing without any side effects. This strategy allows for a local diffusion of biomolecules where the effect is expected. Several kinds of biomolecules have been studied in regenerative medicine. The most popular drugs are antibiotics and growth factors. Antibiotics are widely used in orthopedic surgery to prevent the infection of implants. They are also applied on cutaneous chronic wounds to protect skin from bacterial colonization. Growth factors involved in tissue repair such as PDGF, TGF or NGF are studied to promote nerve or skin regeneration. Different drug delivery systems have been studied to obtain a release of biomolecules that, importantly, is sustained because a rapid diffusion from materials is ineffective in promoting tissue repair. The more sophisticated materials are able to release several biomolecules in a sequential manner to reach the optimal effect. Another approach is the release of therapeutic genes (pDNA) or siRNA to modulate cell phenotype. Drug delivery systems often differ from the native scaffold used for its physical and chemical properties. Generally, this leads to the generation of a composite material with the aim of associating polymers to mimic the native tissue with a drug delivery system.

In this manuscript we aim to present the recent developments of tissue engineered materials studied for bone, skin and nerve regeneration. This choice has been influenced by the importance of these devices in the global market of biomaterials. In addition, we present an overview of the different drug delivery systems developed to deliver the different kind of therapeutic agents.

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2. RECENT DEVELOPMENTS IN TISSUE ENGINEERED DEVICES

2.1. Bone Substitutes

Bone is a vascularized and highly specialized connective tissue which has mechanical and metabolic functions. This tissue is composed of an inorganic phase (70% mineral in the form of hydroxyapatite) and 30% of an organic phase (95% collagen I). Despite its natural capacity to self-heal, bone healing is impossible in the cases of large bone defects caused by trauma or a tumour resection [5]. The gold standard is bone autograft. Nevertheless, harvesting autologous bone from donor sites such as iliac crest is painful and may lead to morbidity of donor sites [6]. Hence, the implantation of biomaterials to treat bone disease or trauma appears promising. Bone substitutes need to have some specifications such as a good reliance on loading and a porous network to favour cell infiltration [7]. Nowadays, the research is towards the association of biomolecules with implants with the aim of enhancing osteointegration, promoting bone tissue repair or preventing infection [6].

Metallic materials were the first to be commercialised to treat bone defects. These devices offer a wide range of mechanical features for load bearing applications (hardness, ductility, formability). Nevertheless, they suffer some limitations such as the lack of biological activity, the absence of porosity and the mismatch in mechanical properties between the host organism and the material. Metallic implants used in the orthopedic field are surrounded by fibrous tissue which can lead to implant failure [8]. Bone-tissue engineering appears as an alternative because it focuses on biomaterials that can favour wound healing while providing mechanical support. After cellular infiltration, the biomaterial has to be remodelled into a neo tissue with properties resembling native bone. Three different approaches have been studied to obtain the ideal bone substitute: (i) the utilization of soft polymers (natural or synthetic), (ii) the application of mineralized materials and (iii) the combination of both to generate composite materials. Natural polymers such as collagen, glycosaminoglycans (GAG)and fibrin have been studied for an application in bone repair. They facilitate cell adhesion and promote osteoinduction [9]. Their disadvantage is their weak mechanical properties [5]. Calcium phosphate ceramics such as tri calcium phosphate (TCP) and hydroxyapatite (HA) have been developed to mimic the mechanical properties of bone and allow for its regeneration. They are osteoconductive as they are colonized by osteoblasts. Then, de novo extracellular matrix is synthesised by cells [10]. Nevertheless, they show some limitations such as their brittleness [6]. Bioactive glasses made of silica and calcium represents an improvement in ceramic technology. They can bond biological tissues, their degradation can be tuned but they are however extremely brittle. Supress Nowadays the trend is to develop composite materials to take advantage of polymer and inorganic-based materials [11, 12]. They can be Collagen/Ha composites or GAG/HA materials promoting osteoblast migration and differentiation [13]. This method has been improved by the development of nanostructured scaffolds which favour bone healing [14]. Nowadays the research is towards the incorporation of cytokines within biomaterials. The most popular loaded biomolecules are BMP-2, VEGF, PDGF and TGF-beta. These cytokines can be coated to Beta-TCP ceramics [15] or can be encapsulated within polymer microspheres [16]. The most effective strategy is to obtain a sequential release of biomolecules with the aim of mimicking the different stages of bone healing. For example, the spatiotemporal release of VEGF and BMP-2 from core shell systems allows for quicker healing [17].

2.2. Scaffolds for Nerve Regeneration

Despite peripheral nerves are able to self-regenerate, inflammation and scarring can prevent their regeneration after a traumatic injury. Surgical nerve coaptation is possible for small gaps (i.e.: 5 mm or less), while larger ones require autologous grafting [18, 19]. However, healthy nerves of the donor site would be affected by this technique [18]. Alternatively, tissue engineered hollow nerve guidance conduits (NGCs) were developed to direct axon growth, prevent excessive scarring and favour nerve regeneration [19]. Nevertheless, NGCs are limited for the treatment of short nerve gaps (less than 4 cm long) and are associated with poor functional recovery [20]. Insufficient levels of regeneration were attributed of inadequate formation of aligned extracellular matrix (ECM) within conduit, i.e. the fibrin cable [21, 22]. The first strategy developed to improve nerve regeneration was the addition of intraluminar guidance cues within neural guide conduits. In the form of fibres, they favour Schwan cells migration and proliferation. Then, Schwann cells produce a matrix made of laminin on which axons migrate. Polymer microfilaments can be used at low density to promote regeneration [23]. Intraluminar structure also includes gels, sponges and films [20]. To favour nerve regeneration, several groups have changed the conduit design. Unlike single channel conduits (such as Neuragen®), multichannel ones are promising to promote nerve regeneration because as they guide the axon growth without any dispersion. Moreover they are biodegradable [24, 25]. To promote the Schwann cells migration, the micro-grooved design and the electrospun deposition of fibers within NCGs have also shown great promise [20]. The addition of topographical guidance structure may require surface modification to increase cell adhesion and migration. This can take the form of full protein or peptide coatings. Laminin is currently used for the surface modification as it is the natural substrate for axon adhesion [22, 26, 27]. Other ECM molecules such as collagen and fibronectin have been used but they enhance the neurite outgrowth much less [28]. The production of ECM proteins is difficult; this explains the development of peptide mimics. They have a high stability, a low immune response, they can be orientated in the conduit and several kind of peptides can be combined to obtain a synergic effect [20, 29]. To improve the regeneration, recent efforts have been toward to creating a conductive environment by the use of external growth factors. Biomolecules have to be used in combination and delivered in a control and sustained manner. To obtain an adequate drug delivery system, several strategies have been developed. The first strategy was the release from polymer coating [30]. One of the most promising techniques is the encapsulation of growth factors within ECM-based matrix places within the laminar of conduits [31]. More recently,

several groups have developed a delivery approach based on the encapsulation of biomolecules within polymer microspheres [32].

2.3. Wound Dressings for Skin Repair

Cutaneous chronic wounds are characterized by an impaired healing six weeks after the injury. The main cutaneous chronic wounds affecting the patients are diabetic foot ulcers venous ulcers and bedsores [33]. The impaired wound healing is characterized by a chronic inflammation. As a consequence, a large breakdown of extracellular matrix occurs, an impaired re-epithelialization and the absence of wound closure [34].

The classic treatment is the debridement of the wound bed to remove necrotic tissue [35]. This procedure is followed by the compression of the wound with a sterile gauze [36]. Sometimes, this technique is inefficient and a wound dressing is required. The ideal wound dressing has to supply an adequate moist environment, absorb exudates, protect against infection, be biocompatible and promote tissue regeneration. In addition, these biomaterials have to be removed from the wound bed without any trauma [35].

Wound dressings can be made from natural or synthetic polymers or a combination of both. Natural polymers are biocompatible, can be biodegradable and sometimes promote wound healing by their interaction with cells. Their main disadvantages are their weak mechanical properties (they often need to be cross-linked by chemical reagents) and their cost. Cross-linking may lead to warrant effects if the chemicals are released within the body. In contrast, synthetic polymers present reproducible and consistent properties, they are cost effective but they are insensitive to physiological degradation by enzymes. Moreover, they do not modulate cell behavior to promote wound healing.

Wound dressings can be classified into four different categories: hydrocolloids, hydrogels, foams and films [35]. Hydrocolloids are semi-permeable films containing hydrophilic particles. Particles are usually made of polysaccharides. These materials have high abilities to absorb exudates and can create a moist environment after swelling. Their main advantage is an ability to promote auto-debridement (auto destruction of necrotic tissue) after exudate absorption [37]. However they cannot be used when the wound is infected and do not favor tissue regeneration. Hydrogels possess several properties required to promote wound healing. First, they maintain a highly moist environment in the wound bed [38]. Second, they promote wound auto-debridment. They are usually made of synthetic polymers such as poly(metacrylate). Hydrogels are not reactive towards biological tissues but they are difficult to handle because of their low mechanical properties. Usually, they require a secondary dressing such as gauze to be maintained in the wound bed. Foam dressings consist of polyurethane foam with high capacity for liquid absorption. In addition, they are protective against infection. However, they do not accelerate tissue repair effectively as they do not promote re-epithelialisation. They are currently used in the management of first stages in chronic wounds. Films are easy to manipulate, semi-permeable to oxygen and water and impermeable for bacteria. Their major drawback is that they cannot absorb exudates.

With the aim of improving skin tissue repair, research orientation is nowadays towards “bioactive dressings”. These dressing are biomaterials that play an active part in the wound healing [38]. The first strategy relies on the utilization of skin substitutes in the wound bed, following a cell therapy approach [39]. Skin substitutes aim to promote sustained healing without rejection. They consist of a scaffold that forms a template for host cell infiltration and physical support to guide cell differentiation and proliferation. They can be made from natural polymers such as collagen (Apligraf®,) or synthetic polymers (Dermagraf®). In addition, they encompass living fibroblasts and keratinocytes, which produce a panel of growth factors. These growth factors promote tissue repair. Skin substitutes are difficult to fabricate and are not cost effective, with a limited shelf-life. A further drawback is the need for their storage in liquid nitrogen to preserve cellular viability. Last, their efficacy on impaired wounds has not so far been clearly shown [40]. The second strategy is based on the application of a medicated dressing on the wound. These modern dressings supress are aimed at delivering biomolecules in a controlled and sustained manner. The drugs encapsulated within the biomaterials are mainly antibiotics or growth factors. Antibiotics are able to prevent or combat infection in the wound bed whereas growth factors can promote angiogenesis, cell migration and proliferation [38]. Growth factors also have a role in the modulation of inflammation. Nevertheless, the presence of cytokines in the wound bed is short-lived because they rapidly diffuse into the body, thereby they do not have their effect within the wound bed [34]. Moreover, the wound environment is filled with proteases that degrade these molecules. Lastly, the main hurdle is to develop a material allowing the drug diffusion over more than one week without initial high burst release to avoid drug toxicity.

3. CONTROLLED RELEASE OF BIOMOLECULES FROM BIOMATERIALS

3.1. Gene Delivery

Gene therapy is considered as a new breakthrough in molecular medicine because it allows the correction of genetic disorders such as hemophilia or adrenoleukodystrophy [41, 42]. It involves the insertion of therapeutic genes in genome of targeted cells to treat the genetic diseases. New therapeutic expectation has recently appeared for the treatment of acquired life threatening diseases caused by genetic deficiencies and abnormalities such as cancer, cardiovascular diseases, metabolic disorders and AIDS [43]. More recently, gene delivery has shown promise for regenerative medicine applications where transitory transgene expression permits the formation of tissues [44, 45]. This strategy aims to substitute drug or protein therapy characterized by the absence of cell targeting and the occurrence of side effects [38]. When a disease causing gene is overexpressed, for example in pathologies characterized by a chronic inflammation, gene silencing therapy via microRNA or siRNA is appropriate to inhibit the translation of the overexpressed gene [46]. In this case, therapeutic genes do not have to go through the nuclear membrane and have their effect inside the cytosol. To treat

numerous pathologies, the sustained and transient production of biomolecules by local cells is required. For this purpose, cell transfection with a plasmidic DNA is necessary to transform them into cell factories. As the nuclear membrane only allows the transport of small molecules, it is established that proliferating cells are easier to transfect [47]. Efficient gene delivery is challenging as DNA cannot enter the cells on its own due to its negative charge. Hence, therapeutic genes have to be associated with transfection reagents. The highest efficiencies are observed when viral vectors are used. These vectors are obtained from viral pathogens, in which the harmful sequences have been replaced by therapeutic genes [48]. The virus's ability to infect cells is used to deliver DNA inside cells (Fig. **1A**). Different viruses are used for gene delivery such as adenoviruses, adeno-associated retroviruses and lentiviruses. Recently, lentiviruses have gained popularity because of their ability to infect dividing and non-dividing cells and to allow the long term production of biomolecules [49]. However, there are still some major concerns in the utilization of viral vectors due to their immunogenicity and oncogenicity [47]. Therefore, research orientation is nowadays towards non-viral vectors such as cationic liposomal formulations and synthetic polymers [50]. Unlike viruses, cationic polymers must have packaging properties to permit DNA delivery into cells. First, polymer must bind DNA and condense it to prevent it from enzymatic degradation by nuclease. Condensation allows for cellular engulfment of polyplexes by endocytosis. Last, the ideal polymer has to promote the endosome escape of polyplexes (DNA/polymer complexes) through a "proton sponge effect" and allows the DNA undressing inside the cytosol [47]. High molecular weight polyethylenimine (PEI) is the most widely studied transfecting reagent with high transfection efficiency and high cytotoxicity [51]. Toxicity is related the PEI concentration in the cytoplasm after DNA release [52]. To lower the PEI toxicity several groups have used low-molecular-weight polymers; but the reduced toxicity was associated with a lower transfection [53]. Another strategy is to favor the polymer biodegradation inside the cell by incorporation of acid-labile linkages within the PEI structure [51]. Several PEI modifications have been tested to lower toxicity, to favor endosomal escape or to prolong the polyplex residence in blood. Polyethylene glycol, chitosan and ligands are the most popular as they increase transfection efficiency while preventing cell toxicity [54, 55]. With PEI modification by ligands, it is possible to release DNA into targeted cells [56]. Natural polymers such as chitosan and polylysine have been tested as transfecting reagents to avoid cytotoxicity. Unfortunately, their application in gene delivery is limited due to their limited ability to condense DNA [57]. Among novel polymers for gene therapy, polyamidoaminedendrimers (PAMAM) proved the most promising because they are highly branched spherical polymers which can strongly condense DNA. Unfortunately, their efficiency and toxicity are generation-dependent [58]. As a consequence, several groups aimed to functionalize PAMAM with PEG or PLL to lower toxicity [59]. Moreover it is possible to graft molecules to target cells [60] (Fig. **1A**). Recently, Newland and collaborators have synthesized a 3D "knot" polymer for non-viral gene delivery. This polymer is a "single cyclized" polymer characterized by the presence of cationic units and PEG units, which exhibit higher transfection abilities than PAMAMs while lowering their toxicity [61].

Another strategy to deliver therapeutic genes is to encapsulate DNA within nanovectors. Poly (lactide-co-glycolic) acid (PLGA) allows for the DNA encapsulation through a double emulsion procedure. Loaded PLGA nanoparticles non-specifically enter the cell and deliver DNA over time [46]. PLGA particles overcome several limitations in miRNA therapy as they protect RNA from degradation [62]. Nonetheless, this strategy is not appropriate for pDNA delivery due to the low release rate of pDNA [58]. Exogenous DNA molecules can be precipitated with calcium phosphate to form DNA hydroxyapatite nanoparticles. When the simulated body fluid is used, it is possible to generate a homogenous population of nanoparticles. Nouri *et al*., have shown high transfection efficiency *in vitro* [63]. Inorganic nanovectors are also of interest for gene therapy. Gao *et al*., have prepared mesoporous silica nanoparticles with ultra-large pores (23 nm) to compact DNA. Plasmidic DNA can diffuse inside the pores, and thereby are protected from nuclease degradation. Moreover, silica has the advantage to be functionalized with moieties or biomolecules, which allows the cell targeting [64, 65].

Viral vectors are typically administrated by local or systemic injection. This can trigger an immune response or the risk of side effects arising from gene expression in an off-targeted region [66]. To overcome these limitations, the association of modified viruses with biomaterials gives new opportunities to obtain a targeted and long-term gene expression. Viral vectors can be encapsulated within materials (microspheres or scaffolds) or immobilized at the materials surface. Microparticles with diameters less than 6 μm may be internalized by phagocytosed by macrophages and smaller ones endocytosed by many kinds of cells. In several cases, the particles could stay at the cell surface and deliver viruses. For example, PLGA microparticles allow for the sustained release of viral vectors [67]. Microporous materials (pores from 10 to 100 μm) are effective only when viruses are encaspulated within material walls. For example the encapsulation within the core of PCL fibers permits their sustained release [68]. Hydrogels are broadly used in tissue engineering to deliver viral vectors. Their synthesis under mild conditions does not impact on vector [69, 70]. Vector diffusion depends on hydrogels porosity, tortuosity, degradability and hydrogels/viruses interaction [49]. Natural polymers allow cell adhesion and migration. In addition, they can be used to entrap viral vectors during their fabrication process. Collagen and fibrin hydrogel can be colonized by host cells after implantation. Hence, the cell transfection occurs within biomaterials. Interestingly, at high fibrin concentration, cell infiltration and vector diffusion are relatively inefficient [69]. Virus modification and biomaterials can also protect vectors from inflammatory, immune response and broad tropism. The first strategy to lower immunogenicity of viral vector is PEGylation, modification with poly-HPMA or coating with polysaccharides. Grafting PEG at the virus surface neutralizes antibodies [71]. Nevertheless, this modification has to be controlled as it tends to decrease cell transfection efficiency [66]. The physical encapsulation within biomaterials has the potential to mitigate pre-existing humoral and cellular immune response. It depends on material porosity

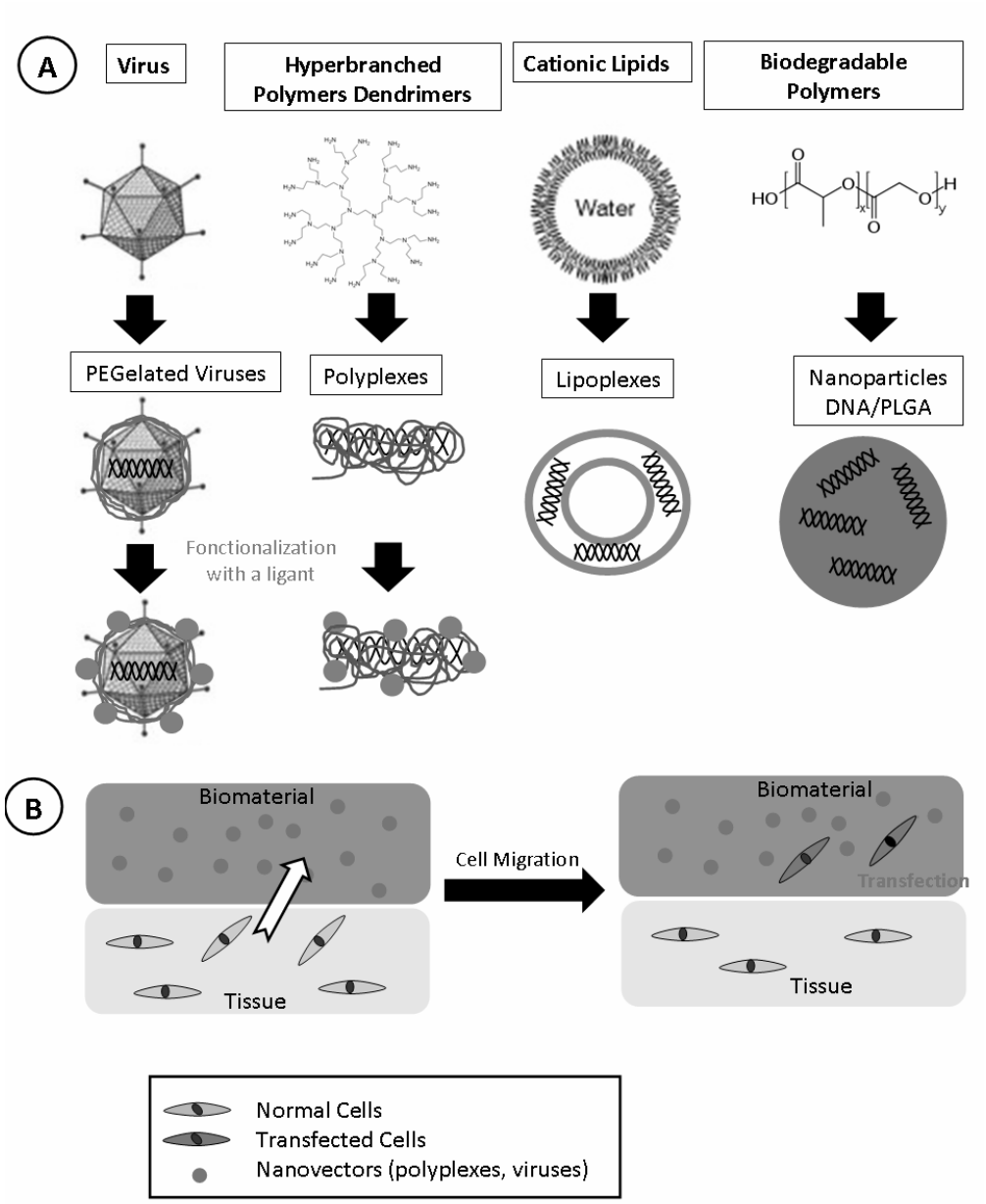

Fig. (1). Gene Delivery from Biomaterials. (**A**): Recent improvement in the fabrication of transfecting reagent. (**B**): Encapsulation of nanovectors within biomaterials. Cell transfection relies on cell infiltration.

and fabrication. Last, virus modification permits the targeting of gene delivery. Adenoviruses can be decorated with fibroblast growth factor -2 after HPMA grafting. As a consequence, virions specifically infected cells expressing FGF-2 receptor [72].

Biomaterials were also used to immobilize polyplexes with the aim to obtain a controlled gene delivery. Natural polymers were first utilized to focus gene delivery within or in the vicinity of materials. Polyplexes were dropped onto collagen sponges or freeze-dried after sponge loading. Unfortunately, gene release occurred within 3 days [73]. Nowadays, efforts are made to design the matrix itself in order to improve transfection efficiency as well as well as to promote tissue repair [74]. Some scaffolds have been designed to allow the sustained release of biomolecules of interest. For example, cutaneous chronic wounds are characterized by a proteolytic environment where cytokines are rapidly degraded [34]. For this reason, the sustained delivery of therapeutic genes within resident cells is required to make them fabricate cytokines. In addition, gene release from biomaterials localizes the effect. In this case, nucleic acid is encapsulated during the scaffold fabrication and controlled release is modulated by the scaffold biodegradation. PLGA was the first polymer for matrix mediated gene delivery as it allows gene release over months [75]. The second approach involves natural polymers such as fibrin, collagen or gelatin which promote cell infiltration (Fig. **1B**). DNA uptake occurs when cells colonize biomaterials. Scaffolds are designed to be sensitive to cellular proteases but have a low porosity to prevent the fast polyplexes release [74]. As gene transfer is correlated with cell proliferation and migration, scaffolds

can be engineered to enhance cell transfection. For example, RGD sequences have been grafted in alginate hydrogels to improve cell transfection [76]. Moreover, the physical state of matrices can also modulate gene delivery as it is known that stiffness reduces cell transfection [76].

3.2. Antibiotic Delivery

Drug delivery systems are designed to improve therapy efficacy as well as patient compliance. Antibiotic delivery systems should present some fundamental characteristics including being inert, biocompatible, with mechanical stability, allow a high drug loading, simple to administer, easy to fabricate and sterilize [77]. Over the last decades, different materials have been developed as drug delivery systems [78, 79]. For instance, poly(D,L-lactide) and poly(glycolide) and their copolymer poly(D,L-lactide-co-glycolide) (PLGA) are the most popular polymers to obtain a controlled drug release [80-82]. Dendrimers are synthetic, highly branched, spherical, monodispersed macromolecules with an average diameter of 1.5-14.5 nm [83]. Liposomes consist of amphiphilic unilamellar or multilamellar membranes of lipids [84]. Alternatively, solid lipid nanoparticles have been used to achieve improved controlled drug release because drug mobility in a solid lipid should be drastically lower than that of liquid oil [85]. In a different approach, inorganic materials have attracted a great deal of interest as functional materials for biomedical applications [86-89].

Nowadays research in the tissue engineering field is focused on the development of bioactive dressings. As mentioned above, these materials are able to control the release of molecules directly at the desired site of action. In this sense, depending on the mechanism involved in the drug release the materials can be swellable or biodegradable. In the first case, swellable materials release antibiotics upon hydration. In the second, the release of the drug occurs during the erosion of the material. Alternatively, in materials that retain their shape the release of the drugs is by diffusion. Nevertheless, in most cases these bioactive dressings possess poor mechanical properties and unsatisfactory drug delivery capabilities since its content is usually released within a short period of time [90]. These lead to serious concerns because the antibiotic could not be present long enough or at the optimum concentration for the necessary time period. Particularly, in these materials an initial burst release is often observed. The burst release can be suppressed by the formation of hydrophobic coatings. With this in mind, Anderson *et al*. developed a polymeric hydrogel system which is able to deliver effective doses of antibiotics over a long period of time. In that work the authors described cross-linked poly(2-hydroxyethyl methacrylate) gels, subsequently surface-modified with octadecyl isocyanate. These materials were able to control the release of norfloxacin by the formation of a hydrophobic rate-limiting barrier [91].

Hence, several physical and chemical properties of the materials should be taken into account to achieve the desired biomedical application. In particular, swelling behavior and crosslink density can be adjusted to control the drug release characteristics of the materials [92-94]. The polypeptide based hydrogels responded to pH-stimulus. In addition their physical properties (*i.e.:* gelling time, mechanical properties, degradation rate) as well as their drug release behavior can be tuned by the modification of the polypeptide composition or the degree of cross-linking [94]. For example, cross-linked collagen sponges are currently used to deliver biomolecules such as growth factors or antibiotics [95, 96]. However, they are not used to treat infections in open wounds such as chronic ulcers because the entire dose of biomolecules is released within a 2 hour period [97, 98]. For the treatment of cutaneous chronic wounds, several strategies have been adopted with the aim of controlling the biomolecules delivery within the injured tissue.

Alternatively, varying polymer concentration was also identified as a valuable alternative to tune both mechanical stability and release kinetics. In this sense, collagen matrices with concentrations ranging from 5 to 40 mg mL^{-1} were recently reported. It was shown that the mechanical properties and resistance against collagenase digestion increase in concentrated collagen matrices. Moreover, after subcutaneous implantation in rats, concentrated collagen matrices exhibited high stability and biocompatibility. Indeed, dense matrices at 40 $mg.mL^{-1}$ loaded with ampicillin inhibited bacterial growth over 3 days [99] (Fig. **2A**). It is worth mentioning that with the aim of delaying and controlling the release of antibiotics in the wound bed, other attempts have been made using synthetic polymers such as poly-caprolactone in the form of meshes or composites [100]. The use of 3-dimensional polycaprolactone-tricalcium phosphate (PCL-TCP) mesh for the delivery of gentamicin sulphate efficiently eliminate bacteria within 2 h and demonstrate low cytotoxicity [101].

More recently, the incorporation of nanoparticles or fillers in hydrogels contributed to fulfill the requirements to enhance mechanical stability and work as a reservoir for the controlled release of antibiotic agents [102]. The combination with a drug delivery system, especially, is required to control and delay the release. Polymer-based nanoparticles have been widely employed with the aim of achieve a controlled release of antibiotics. In this sense, antibiotic loaded poly-(lactic-co-glycolic) acid (PLGA) nanoparticles were incorporated in collagen sponges [103]. Alternatively, extended antibacterial activity over 7 days was observed in silica-collagen nanocomposites. These nanocomposites can be considered as promising biological dressings as they can play an active part in the wound healing process [104, 105] because: i) they create a suitable environment to promote healing [38] and ii) the combination of a high collagen concentration with silica nanoparticles improves hydrogel mechanical stability and handling (Fig. **2B** and **2C**) [106]. The combination of these distinct constituents in a single scaffold proved an effective way to develop drug delivery system. Recently, a hydrogel-nanoparticle hybrid scaffold that provides a chemically-defined, remotely-triggerable and on-demand release of small molecule drugs was reported. Upon photo-irradiation, the activation of the photo-triggerable compound is designed to initiate a series of intramolecular chemical rearrangements, which would cleave the covalently-bound drug and release it from the hydrogel [107]. Finally, the integration of different materials offers the possibility to tune the desired mechanical properties, drug release mechanism and thus the desired drug release patterns.

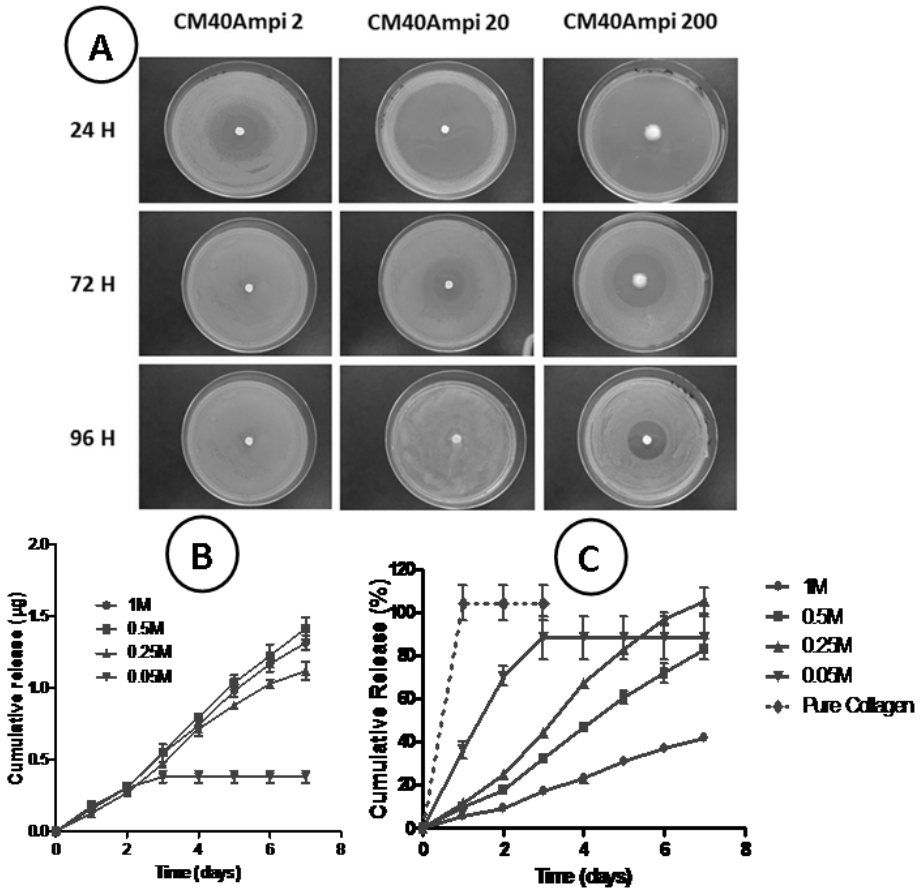

Fig. (2). Gentamicin release from silica-collagen nanocomposites with various silica contents: (**A**) Inhibition of bacterial growth by ampicillin loaded dense collagen matrix (40 mg mL^{-1}) (ref. [99]–reproduced with permission from The Royal Society of Chemistry (RSC)). (**B**) cumulative released dose and (**C**) cumulative released percentage of initial loading calculated for each nanocomposite composition. The dashed line on (**B**) shows gentamicin release from collagen hydrogels. Results are expressed as mean± SD from triplicate experiments (ref. [106]–reproduced with permission from The Royal Society of Chemistry (RSC)).

3.3. Growth Factors Delivery

Tissue engineering and regenerative medicine aim to regenerate a functional tissue through the use of an appropriate combination of "tools" [108]. Recently, a better understanding of the biological processes underlying tissue repair led to the development of new approaches which consist in the combination of appropriate cells and biodegradable scaffolds in which specific environmental cues, such as growth or adhesive factors are encapsulated [109].

Growth factors are small proteins known to play a central role in information transfer between a wide range of cells and their extracellular matrix. These cytokines stimulate endogenous repair mechanisms by providing the right signals to cells and thereby leading to an accelerated healing of damaged or defective tissues [110].

Exogenous growth factors that are injected in solution into the wound site are generally not effective. This is because of a rapid *in vivo* clearance occurs due to the diffusion away from wound locations or because of enzymatic digestion or inactivation. Thus, there is an increasing need to control the loaded dose and the release kinetic of growth factors released during tissue healing. In particular, the ability to control the release of growth factors through covalent or non-covalent strategies has been used in various tissue engineering applications [111, 113]. Release studies showed a more controlled delivery when proteins were incorporated during the scaffold preparation than when the protein has been loaded in a second step (*ex situ* process) [114].

Sophisticated delivery systems offer the ability to deliver multiple growth factors with independently tunable kinetics has become the focus of intense research over the recent years [115, 116]. A series of self-reinforcing hyaluronan hydrogels was developed to improve mechanical properties and protein sustained delivery [117]. Furthermore, hybrid scaffolds of collagen and poly(lactic-co-glycolic acid) microbeads were prepared by introducing insulin-releasing poly(lactic-co- glycolic acid) microbeads into collagen porous scaffolds [118]. Subsequently the singular and combined effects of bone morphogenetic protein (BMP-2), platelet derived growth factor (PDGF-BB), and collagen-GAG (CG) biomaterial membrane stiffness on the bioactivity and gene expression were examined [119].

Alternatively, a bilayer polymer film comprising a drug reservoir layer and a supporting layer is fabricated by spin-coating poly(lactic-co-glycolic acid) (PLGA) on top of a

layer-by-layer assembled film of poly(-amino esters) (PAE), alginate sodium (ALG), and recombinant human basic fibroblast growth factor. The films that adhere strongly to tissue and can deliver therapeutic agents in a controlled manner [120]. Indeed, bone morphogenetic protein-2 (BMP-2) delivered from a bilayer scaffold of segmented polyurethane/ polylactic- co-glycolic (SPU/PLGA) successfully promoted cartilage repair [121]. In addition, Min *et al*., reported a polymer-based coating, obtained by using a water-based layer-by-layer (LbL) approach, as a biomimetic implant surface that provides staggered release of an antibiotic followed by that of an active growth factor for orthopedic implant applications [122].

Growth factor encapsulation into microparticles was also reported. Insulin like-growth factor-1 (IGF-1) was encapsulated into microparticles fabricated using chitosan as a natural polymer cross-linked with tripolyphosphate [123]. Chitosan microparticles with a size range between 20 and 70 μm were functionalized by carbodiimide followed by the immobilization of an anti-PDGF-BB antibody and used to immobilize the platelet derived growth factor (PDFG-BB). The presence of immobilized cytokines gives to this material a biological functionality towards control on cell behavior [124]. Recently, a multilayered, mineral coated microparticle platform is developed for tunable dual bone morphogenetic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF) delivery [115]. In a different approach, murine VEGF164 was fused to a sequence derived from α2-plasmin inhibitor ($\alpha2\text{-PI}_{1\text{-}8}$) that is a substrate for the coagulation factor fXIIIa. This covalent crosslinking into fibrin hydrogels only permitted the VEGF164 release after enzymatic cleavage. This strategy ensured sustained and tunable release of VEGF [125]. Last example, Lu *et al.* have shown it was possible to load Insulin-like growth factor-1 (IGF-1) and bone morphogenetic protein-2 (BMP-2) into gelatin microparticles. Loaded microparticles were then embedded within an oligo(poly(ethylene glycol) fumarate) hydrogel matrix to deliver these growth factors to promote the regeneration of osteochondral tissue [126].

4. CONCLUSIONS

To date, a broad diversity of polymers, including natural based materials (*i.e.*: chitosan, alginate, and collagen) and synthetic ones (i.e.: poly D,L-lactide, poly glycolide, poly 2-hydroxyethyl methacrylate) were successfully applied in the design of biomaterials. The complex properties resulting from the combination of polymers with therapeutic agents could provide advantages over existing biomaterials. In particular, the interaction between the different components of medicated biomaterials, *i.e* scaffolds and biomolecules as well as the behavior of devices within biological systems needs to be precisely analyzed, since their interaction will determine the resulting function performance. Therefore the development of biological substitutes that restore, maintain or improve tissue function requires the contribution from various research areas (*i.e.*: chemistry, biology, pharmacy, physics) to understand and further develop these advanced biomaterials that should be considered as a true multidisciplinary field of research.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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