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Adsorption and release kinetics of growth factors on barrier membranes for guided tissue/bone regeneration: A systematic review

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Running title: Adsorption and release of growth factors

Highlights

- -Most membranes functionalized with growth factors were from natural origin, obtaining better results that synthetic membranes.

- -The majority of the studies showed that membranes release greater quantity of growth factors the first 24 hours, compared to the following time points.

- -The first phase is characterized by a rapid release, whereas during the second phase this release was much slower.

Abstract

Objectives: Guided bone / tissue regeneration (GBR/GTR) procedures are necessary to improve conditions for implant placement. These techniques in turn can be enhanced by using growth factors (GFs) such as bone morphogenetic protein (BMP-2) and platelet-derived growth factor (PDGF) to accelerate regeneration. The aim of the present systematic review was to evaluate the GF loading and release kinetics of barrier membranes.

Study design: A total of 138 articles were screened in PubMed databases, and 31 meeting the inclusion criteria were included in the present systematic review.

Results: All the articles evaluated bio-resorbable membranes, especially collagen or polymer-based membranes. In most studies, the retention and release kinetics of osteogenic GFs such as BMP-2 and PDGF were widely investigated. Growth factors were incorporated to the membranes by soaking and incubating the membranes in GF solution, followed by lyophilization, or mixing in the polymers before evaporation. Adsorption onto the membranes depended upon the membrane materials and additional reagents such as heparin, cross-linkers and GF concentration. Interestingly, most studies showed two phases of GF release from the membranes: a first phase comprising a burst release (about 1 day), followed by a second phase

characterized by slower release. Furthermore, all the studies demonstrated the controlled release of sufficient concentrations of GFs from the membranes for bioactivities.

Conclusions: The adsorption and release kinetics varied among the different materials, forms and GFs. The combination of membrane materials, GFs and manufacturing methods should be considered for optimizing GBR/GTR procedures.

Abbreviations: COL; collagen, IGF; insulin-like growth factor, FN; fibronectin, BSA; bovine serum albumin, mSIS; mineralized decellularized matrix from the small intestinal submucosa, HPLC; high performance liquid chromatography, FGM; functionally graded membrane, MTZ; metronidazole, PDLLA; Poly(L-lactide-co-D/L-lactide), PPCM; porcine pericardium collagen membranes, PDCM; porcine dermis-derived collagen membranes, SF; silk fibroin, PLLACL; poly (L-lactide-co-caprolactone), DFO; deferoxamine, DMOG; dimethyloxalylglycine, PHD; prolylhydroxylase, PSEC; platelet secretome, SDF-1; stromal cell-derived factor-1, ADM; acellular dermal matrix, CLSM; confocal laser scanning microscopy, PBS; phosphate buffer saline, PCL; polycaprolactone, FS; free-standing, PCL; polycaprolactone, PLGA; poly(lactic-coglycolic acid), β-TCP; beta-tricalcium phosphate, L-MIM; L-mimosine, HyA; hyaluronic acid, PCL; poly(caprolactone), PEG; poly(ethylene glycol), PDO; poly(dioxanone), nBG; nano-bioactive glass, α-MEM; α-minimal essential medium, vitrigel; stable collagen gel membrane prepared from vitrified type I collagen, SMCC; succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, PLLA; poly(L-lactide), TCP; tricalcium phosphate, PGA; polyglycolic acid.

Declarations of interest: none
Key words: growth factors, barrier membranes, guided bone regeneration, guided tissue regeneration.

1. Introduction

Periodontal regenerative techniques require the use of barrier membranes for guided tissue/bone regeneration (GTR/GBR) (Renvert & Persson, 2016; Scannapieco & Cantos, 2016). Such regeneration procedures are based on the concept of enhancing the formation of bone and/or periodontal tissues by excluding unwanted epithelial and connective tissue ingrowth into hard tissues by means of a barrier membrane (Buser et al., 1998; Dimitriou, Mataliotakis, Calori, & Giannoudis, 2012). Since their introduction in the early 1980s, different types of biomaterials and techniques have been developed to improve their effectiveness, rigidity and biocompatibility (Dahlin, Linde, Gottlow, & Nyman, 1988; Dimitriou et al., 2012; Jimenez Garcia, Berghezan, Carames, Dard, & Marques, 2017). Furthermore, a wide variety of approaches including bone grafting, osteoconductive/inductive materials, protein mixtures, exogenous growth factors, cell-based technology and gene therapy have been investigated to further develop GTR/GBR procedures

(M. C. Bottino et al., 2012). The first generation of barrier membranes consisted of non-resorbable membranes including expanded polytetrafluoroethylene (e-PTFE) membranes, titanium reinforced ePTFE, high density PTFE and titanium meshes (Liu & Kerns, 2014; Sheikh et al., 2017). These membranes require a second surgical procedure for removal, and have a higher risk of exposure to the oral environment, thereby increasing the risk of secondary infection and disturbing the regeneration process (Dimitriou et al., 2012; Selvig, Kersten, Chamberlain, Wikesjo, & Nilveus, 1992; Simion, Baldoni, Rassi, & Zaffe, 1994; Zitzmann, Naef, & Schärer, 1997).

In the last decades, a variety of synthetic and natural resorbable membranes have been introduced. In many cases these membranes are able to avoid the soft tissue complications of non-resorbable membranes (Cortellini, Pini Prato, & Tonetti, 1996; Sheikh et al., 2017; H. L. Wang, O'Neal, Thomas, Shyr, & MacNeil, 1994; Yukna & Yukna, 1996). Although the durability of the barrier effect decreases over the healing period, these membranes allow a single-step surgical procedure, which decreases patient morbidity by lowering the risk of membrane exposure (Dimitriou et al., 2012; Greenstein & Caton, 1993). Most of the commonly used resorbable membranes are made of synthetic polymers like polylactic acid (PLA) and polyglycolic acid (PGA), or biopolymers such as collagen or chitosan (J. Wang et al., 2016). Collagen-based membranes are widely used for clinical procedures thanks to their ability to promote progenitor cell adhesion, chemotaxis and hemostasis, experiencing physiological degradation and presenting low immunogenicity (Bunyaratavej & Wang, 2001). Given the need in some cases to improve the mechanical properties of resorbable collagen membranes, physical and chemical enzymatic processes have been developed; such processes are known to promote cross-linking (Bozkurt et al., 2014; Jimenez Garcia et al., 2017).

In order to improve the barrier membrane characteristics, other processes such as the incorporation of growth factors (GFs) have recently been developed. Growth factors are able to accelerate the healing process and therefore enhance tissue regeneration. In the dental field, GFs including bone morphogenetic protein BMP-2 and platelet-derived growth factor (PDGF) are often used to promote tissue regeneration (Kaigler et al., 2011). Recombinant human (rh) BMP-2 is considered the most actively studied recombinant protein for bone regeneration, and has been widely used in clinical practice across many fields of medicine (Jung et al., 2003; Matin, Nakamura, Irie, Ozawa, & Ejiri, 2001; Nakashima & Reddi, 2003). These GFs have been shown to increase the proliferation and differentiation of mesenchymal cells into bone-forming osteoblasts and to improve the speed and quality of new bone formation (Miron, Saulacic, Buser, Iizuka, & Sculean, 2013). Many studies have evaluated the BMP-2 bone-inducing capacity in a number of dental treatments such as alveolar crest augmentation, sinus lifts, socket preservation techniques, immediate implant placement and other complex GBR procedures (Boyne et al., 1997; Wikesjö et al., 2003; Wikesjö et al., 2004). Platelet-derived growth factor (PDGF) is a recombinant growth factor that

has been approved by the United States Food and Drug Administration (FDA) and is known for its potent chemotactic and mitogenic effect upon a wide variety of cell types, including gingival and periodontal ligament fibroblasts, cementoblasts and osteoblasts (Nevins, Camelo, Nevins, Schenk, & Lynch, 2003).

Strategies based on the combination of barrier membranes and GFs are believed to accelerate the effect of GTR/GBR procedures. Growth factors have short biological half-lives, localized actions and rapid local clearances. To overcome these drawbacks, it has been well documented that the GF carrier systems play a key role in determining GF bioactivity. In recent years there has been a strong increase in research centered on the appropriate carrying material for fully controlled and optimal GF release (Michalska, Kozakiewicz, Bodek, & Bodek, 2015; Michalska, Kozakiewicz, & Bodek, 2008). Collagen-based membranes and other membranes have been proposed as key biomaterials capable of securing sustained release of GFs over a period of time, and of affording ideal release kinetics of GFs (H.-y. Zhao et al., 2015). Accordingly, the purpose of this systematic review was to investigate the influence of barrier membranes used in GBR and GTR techniques in terms of loading and release of the most commonly used GFs in regenerative dentistry.

2. Methods

2.1 Protocol development

A protocol including all aspects of a systematic review methodology was developed prior to starting the review. This included definition of the focused question, a defined search strategy, study inclusion criteria, determination of outcome measures, screening methods, data extraction and analysis and data synthesis.

2.2 *Defining the focused question*

The following focused question was defined: ”What are the absorption and release kinetic properties of various barrier membranes used for guided tissue and bone regeneration?”

2.3 *Search strategy*

Using the MEDLINE database, a literature search was for articles published up to and including 31 May 2018. Combinations of several search terms were applied to identify appropriate studies (Table 1). Reference lists of review articles and of the articles included in the present review were screened. Finally, a manual search was made of the *Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontal Research, Journal of Periodontology* and *International Journal of Periodontics and Restorative Dentistry*.

2.4 *Criteria for study selection and inclusion*

Study selection only considered articles published in English and describing *in vitro*, animal and

human clinical studies on the ability of barrier membranes to adsorb and release growth factors (barrier membranes, GBR membranes, GTR membranes, and growth factors/proteins and combinations thereof). Membranes could include chitosan, chitosan-collagen and polyglycolic acid (PGA) membranes, natural collagen membranes of human, bovine, porcine, pericardial and dermal origin, polytetrafluoroethylene (PTFE) membranes, and bone lamina membranes.

2.5 *Outcome measure determination*

The primary outcome of interest was the amount in volume, weight or percentage of growth factor adsorption of membranes as well as the time required for full protein loading/adsorption. Thereafter, the release kinetics in volume, weight or percentage of growth factor release over time was characterized.

2.6 *Screening method*

The titles and abstracts of the selected articles were independently screened by two reviewers (Y.A.-M. and M.F.-K.). Screening was based on the question: “What membranes have been utilized to determine the absorption and release kinetic properties of various barrier membranes used for guided bone and tissue regeneration?“ Full text articles were retrieved if the response to the screening question was “yes” or “uncertain”. The level of agreement between reviewers was determined by kappa scores. Disagreement regarding inclusion was resolved by discussion between authors. In the case of necessary missing data, the authors of the studies were contacted.

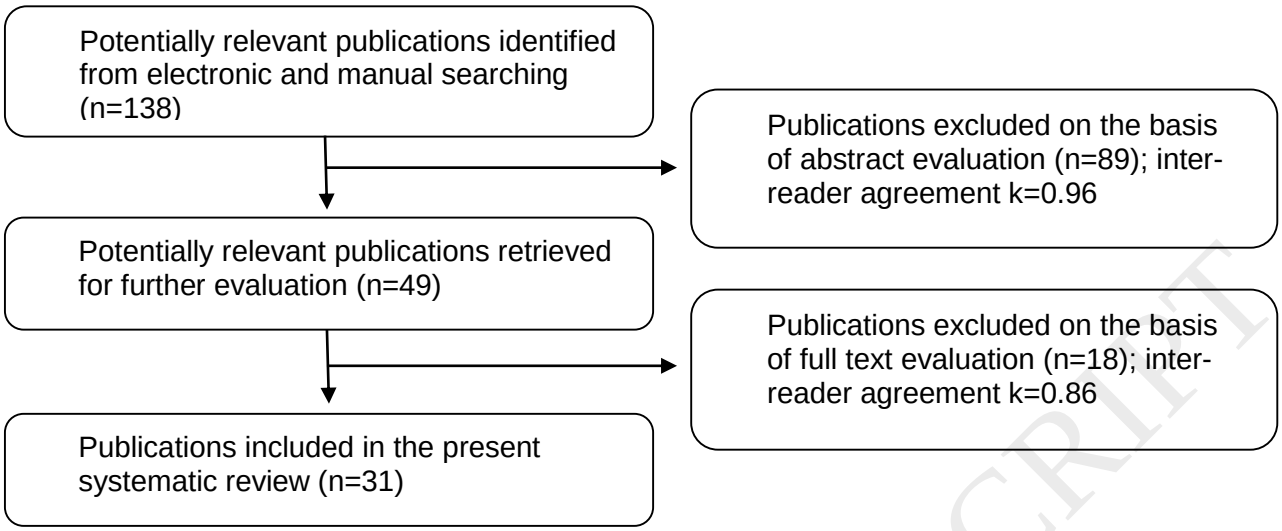
2.7 *Data extraction and analysis*

The following data were extracted: general characteristics (authors, year of publication); membrane characteristics (membrane source, type); evaluation characteristics (weight, volume, period, outcome measures); methodological characteristics (study design, methodological quality); and conclusions. Because of the heterogeneity of the included studies (study design, *in vitro* versus animal studies, investigated parameters, materials used, evaluation methods, outcome measures, observation periods), no mean differences could be calculated, and hence no quantitative data synthesis or meta-analysis could be performed. Consequently, data were extracted from the reviewed articles and summarized in separate tables based upon the various types of biomaterials and outcome measures employed.

Table 1. Search terms used to identify the relevant studies.

Search terms
"Guided Tissue Regeneration" OR "GTR" OR "Guided Bone Regeneration" OR "GBR" OR "Bone Regeneration" OR "Periodontal Regeneration" OR "Bone Tissue Regeneration" OR "Bone formation" OR "Osteogenesis" OR "Osteogenic regeneration"
AND
"Barrier Membrane" OR "Membrane" OR "Barrier" OR "Collagen Membrane" OR "Chitosan Membrane" OR "Chitosan-Collagen Membrane" OR "PGA Membrane" OR "Poly-Glycolic Acid Membrane" OR "Human Membrane" OR "Natural Membrane" OR "Bovine Membrane" OR "Porcine Membrane" OR "Pericardium Membrane" OR "Dermis Membrane" OR "PTFE Membrane" OR "Bone Lamina Membrane" OR "Cross-linked Membrane" OR "Non-cross-linked Membrane" OR "Dura Membrane"
AND
"Absorption" OR "Adsorption" OR "Absorb" OR "Adsorb" OR "Absorbed" OR "Adsorbed" OR "Adhesion" OR "Release" OR "Released"
AND
"Growth Factor" OR "Bioactive Protein" OR "Platelet-Derived Growth Factor" OR "PDGF" OR "Bone Morphogenetic Protein" OR "BMP" OR "Enamel Matrix Derivative" OR "EMD" OR "Emdogain" OR "Enamel Matrix Protein" OR "EMP" OR "Fibroblast Growth Factor" OR "FGF" OR "Platelet Rich Plasma" OR "PRP" OR "Growth and Differentiation Factor" OR "GDF" OR "Transforming Growth Factor" OR "TGF" OR "Vascular Endothelial Growth Factor" OR "VEGF"

Figure 1. Flow chart of the screened relevant publications.



3. Results

3.1. General results

The PubMed search yielded a total of 138 titles considered potentially relevant. In the second phase of study selection, 49 articles were retrieved for further evaluation and total of 31 articles were selected. Studies containing direct *in vitro* and *in vivo* assays of adsorption and release kinetics of GFs from membranes were included in our study. Publications that only examined the bioactivity of GF-combined membranes such as cell responses or bone formation were excluded.

3.2. Membrane material and manufacturing procedure

All studies targeted bio-resorbable membranes, and most of them tested resorbable collagen-based membranes. The following commercially available collagen barrier membranes were utilized in the selected studies: BioGide® (Geistlich Pharma AG, Wolhusen, Switzerland) (Edelmayer, Al-Habbal, Pensch, Janjic, & Agis, 2017; Hamid, Pensch, & Agis, 2015; Mozgan et al., 2017), OsseoGuard® (non-crosslinked bovine type I collagen, Biomet 3i, Warsaw, IN, USA) (Takayama et al., 2017), acellular dermal matrix (ADM) membranes (ZhengHai Biotechnology Co. Ltd., Yantai, Shandong, China), native porcine pericardium collagen membrane (Jason® membrane, Botiss, Zossen, Germany) and porcine dermis collagen membrane (Mucoderm®, Botiss) (Fujioka-Kobayashi et al., 2017). Furthermore, collagen could also be combined with the other polymers creating hybrid membranes. Ho et al. introduced a functionally graded membrane (FGM) with a core layer of collagen (BioMend® Extend®, Zimmer Biomet Inc., Warsaw, IN, USA), which encapsulated metronidazole (MTZ) in the nanofibers of the outer surface to reduce the risk of bacterial infection. Furthermore, it incorporated PDGF in the nanofibers of the inner surface in order to enhance osteogenesis (Ho et al., 2017). Michalska et al. compared the effect of homogenous fibrin, collagen and composite fibrin-heparin and a fibrin-collagen membrane (Michalska et al., 2015). Zhao et al. in turn tested type I collagen gel and collagen vitrigel membranes. Vitrigel is a stable collagen gel membrane prepared from vitrified type I collagen. This membrane not only increases the mechanical strength and maneuverability of the collagen material but also slows the biomolecules release rate (J. Zhao et al., 2009). The composite membrane, mineralized decellularized matrix from the small intestinal submucosa (mSIS) mainly comprised collagen fibers (T. Sun et al., 2018). The pure SIS membrane was used for GBR, providing an osteogenic remodeling microenvironment *in vivo* (Elgali et al., 2016; T. Sun et al., 2018).

Chitosan and chitosan derivative materials were also used due to their good biocompatibility. Four studies investigated chitosan-based membranes: chitosan nanofibrous membrane (Park et al., 2006), chitosan and chitosan–silica xerogel hybrid membranes (E. J. Lee & Kim, 2016), double-layered alginate-chitosan polymer films (Michalska et al., 2008), and chitosan/alginate free-standing (FS) membrane (Caridade et al., 2015).

Other synthesized resorbable polymers were also designed as GBR/GTR membranes: poly(ethylene glycol)/poly(caprolactone) (PEG/PCL) membrane (Zhu et al., 2013), poly(dioxanone) (PDO) membrane

(Boehringer Ingelheim, Germany) (Kim, Lee, Kim, Koh, & Jang, 2012), and PLGA membrane (GC membrane®, GC Corporation, Tokyo, Japan) (Ono et al., 2013). Other membranes were introduced such as polycaprolactone (PCL)/gelatin composite fiber meshes (J. H. Lee, Lee, Cho, Kim, & Shin, 2015), poly(L-lactide) and tricalcium phosphate (PLA/TCP) (S. J. Lee et al., 2001; Y. M. Lee et al., 2003), PLA–alginate membrane (Milella et al., 2001) and PLA porous membrane (C. P. Chung, Kim, Park, Nam, & Lee, 1997; Park, Ku, Chung, & Lee, 1998). In order to improve cell migration and GF entrapment potential, composite materials afforded the necessary microenvironment. The coaxial electrospun fibrous membranes from SF/PLLACL enabled the loading of GFs in a core structure and exhibited a good three-dimensional core-shell structure, with suitable porosity and physicochemical properties (Yin et al., 2017).

Fibrous glass membrane constructed from unwoven glass fibers (Advantec Co., Tokyo, Japan) (Takita et al., 2004) and the membrane form of the collagen/nano-bioactive glass (nBG) hybrid (Hong et al., 2010) were also examined. Bioactive glass materials aimed to afford osteoconductive potential on barrier membranes.

3.3. Growth factors for GTR/GBR

The adsorption and release kinetics of osteogenic GFs from the membranes were investigated. Transforming growth factor β (TGF-β) (Michalska et al., 2008; Milella et al., 2001), BMP-2 (Caridade et al., 2015; E. J. Chung, Chien, Aguado, & Shah, 2013; Du et al., 2017; Fujioka-Kobayashi et al., 2016; E. J. Lee & Kim, 2016; Y. M. Lee et al., 2003; Ono et al., 2013; Park et al., 2006; Shim et al., 2014; Takita et al., 2004; J. Zhao et al., 2009; Zhu et al., 2013), BMP-9 (Fujioka-Kobayashi et al., 2016), a short peptide P28 (Cui et al., 2016; Tingfang Sun et al., 2018), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) (Ho et al., 2017; Takayama et al., 2017), -AB (Michalska et al., 2008) -BB (C. P. Chung et al., 1997; S. J. Lee et al., 2001; Michalska et al., 2015; Park et al., 1998), platelet concentrate, secretome of washed platelet (washed PSEC) and secretome of unwashed platelet (unwashed PSEC) (Mozgan et al., 2017), fibroblast growth factor (FGF)-2 (Du et al., 2017; Hong et al., 2010; J. H. Lee et al., 2015), FGF-18 (Imamura et al., 2018), stromal cell-derived factor-1 (SDF-1) (Takayama et al., 2017) and insulin-like growth factor (IGF)-1 (Sadeghi et al., 2018) were tested. Almost one-half of the studies focused on BMP-2.

Furthermore, prolylhydroxylase (PHD) inhibitors, dimethyloxalylglycine (DMOG) and L-mimosine (L-MIM), and deferoxamine (DFO) were applied to enhance VEGF production (Edelmayer et al., 2017; Hamid et al., 2015).

3.4 Adsorption potential of GFs

Most studies incorporated GFs to the membranes by soaking and incubating the latter in GF solutions for defined periods. The shortest incubation time was 5 minutes (Fujioka-Kobayashi et al., 2017), while the longest was 24 hours (J. H. Lee et al., 2015; Ono et al., 2013; T. Sun et al., 2018). Fujioka-Kobayashi et al. coated BMP-2 and BMP-9 onto PPCM and PDCM collagen membranes with incubation time

of 5 minutes (Fujioka-Kobayashi et al., 2017). Interestingly, approximately 90% of both BMPs were incorporated to both natural collagen membranes (Fujioka-Kobayashi et al., 2017). In the study of Milella et al., TGF-β solution (PBS containing 0.2% BSA) was added to the cross-linked alginate membranes and incubated for 4 hours at 37ºC (Milella et al., 2001). Park et al. immobilized BMP-2 by incubating a (SMCC)-linked chitosan membrane with succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate for 10 hours at 4ºC (Park et al., 2006). The chemical binding achieved a covalent attachment of the target molecule to the solid surface, resulting in an irreversible bond with high levels of surface coverage. Hong et al. prepared the membranes by dropping FGF-2 in α-MEM onto the membrane and incubated it for 2 hours at room temperature (Hong et al., 2010). The FGF2 adsorbed onto the membrane was estimated to be 4.68, 39.5 and 57.3 μg/ml with the initial treatment of 10, 50 and 100 μg/ml, respectively (Hong et al., 2010).

Lee et al. immersed heparinized PCL/gelatin composite fiber membranes in FGF-2 solutions for 24 hours at room temperature. An amount of 21.95 ± 4.5% of the FGF-2 was physically adsorbed onto the PCL/gelatin fiber membranes without heparinization upon reacting with 100 ng/ml of FGF-2. In contrast, the immobilization yield of FGF-2 bound to the heparinized membranes was significantly increased to 58.60± 2.5% on reacting with 50 ng/ml of FGF-2. Moreover, heparinized membranes with the same concentration (100 ng/ml) showed a slightly higher immobilization yield (29.25± 7.6%) than the non-heparinized membranes (J. H. Lee et al., 2015). Lee et al. investigated the adsorption potential of BMP-2 on two different membranes: chitosan membrane and chitosan-silica xerogel hybrid membrane using CLSM and GFP labeled BMP-2 (E. J. Lee & Kim, 2016). Both membranes showed uniform BMP-GFP adhesion in a dose-dependent manner, and the hybrid membrane was associated with a higher level of protein adhesion than the pure chitosan membrane (E. J. Lee & Kim, 2016).

The other major method for incorporating GFs into the membranes was lyophilization (Du et al., 2017; Hamid et al., 2015; Y. M. Lee et al., 2003; Takita et al., 2004). Both Edelmayer et al. and Mozgan et al. utilized BioGide® collagen membranes soaked in PHS inhibitors and PSEC, respectively, and then lyophilized them with a freeze dryer (Edelmayer et al., 2017; Mozgan et al., 2017).

Furthermore, the GFs were incorporated into the synthesized polymer solutions such as PLA, PGA during membrane manufacture. Lee et al. manufactured the PLLA/TCP membrane with the incorporation of PDGF-BB into the PLA solution by adding TCP to PLA with a 50:50 (w/w) ratio to polymer weight (S. J. Lee et al., 2001). For composite membranes of more than two resorbable polymers, GFs were mixed with one of these polymer solutions and the other polymers were combined afterwards. Ho et al. introduced the functional PDLLA surface layers by electrospinning technology (Ho et al., 2017). The nanofibers encapsulating 3% metronidazole (MTZ) and PDGF in PDLLA were electrospun and deposited on the surfaces of the core layer collagen membrane (Ho et al., 2017). Shim et al. manufactured PCL/PLGA/β-TCP composite membranes (Shim et al., 2014). The PCL/PLGA/β-TCP fibers and collagen/BMP-2 solution were separately dispensed into each layer by a 3D printer (Shim et al., 2014). Equal volumes of 0.5% acid-

solubilized type I collagen solution and BMP-2 solution were mixed and gelatinized, vitrified and rehydrated (J. Zhao et al., 2009). The two studies by Park et al. and Chung et al. used the coating method involving PDGF-BB-dissolved PLA methylene chloride–ethyl acetate solutions on PGA meshes (C. P. Chung et al., 1997; Park et al., 1998).

3.5 Release kinetics of GFs

Most studies confirmed that the membranes showed sustained release of GFs over time. The first phase was characterized by rapid release, while release during the second phase was much slower. Lee et al. found that in the first phase (day one), the chitosan and chitosan–silica xerogel hybrid membranes released larger amounts of BMP-2 compared to the pure chitosan membrane. However, after day one, both membranes released similar amounts of BMP-2 into the fresh media at the prescheduled release time (E. J. Lee & Kim, 2016). Caridade et al., over a one-month observation period, demonstrated that the 5-20% burst-release trends were similar to those observed in the first four hours, and that this was followed by continuous release until a plateau was reached (Caridade et al., 2015). In addition, this burst release was systematically higher for the low crosslinking versus the high crosslinking membranes (Caridade et al., 2015). Shim et al. likewise reported that approximately 25.5% of the total BMP-2 in the membranes was released within 24 hours (Shim et al., 2014). Thereafter, sustained release was observed for up to 28 days, with 47.2% release of the total BMP-2 on day 28 (Shim et al., 2014). Mozgan et al. found the release of total protein levels, PDGF-BB levels, and TGFβ-1 levels to be higher in the first hour, followed by a decrease suggesting that the majority of growth factors were washed out in the first hours of incubation (Mozgan et al., 2017). The release of BMP-2 from the PLLA/TCP membrane also occurred in two phases: an initial immediate phase on the first day and a second phase thereafter (Y. M. Lee et al., 2003). Approximately 70% of the BMP-2 was released during the first day, and BMP-2 was consistently released at a rate of 7-10 ng/day for up to four weeks (Y. M. Lee et al., 2003). A similar trend was observed with the majority of DMOG and L-MIM, which were released from the collagen membrane (BioGide) within the first hours - reaching the highest levels in the first hour (Hamid et al., 2015). Chung et al. observed a relatively long period of GF release, showing approximately 17% of the total incorporated BMP-2 to be released by day 49 (E. J. Chung et al., 2013). These authors suggested that the faster release rate after day 21 might be due to degradation and changes in the membrane structure over time (E. J. Chung et al., 2013).

The materials of the membranes also influenced GF release kinetics. A different trend in GF release was shown by Zhao et al., with most of the BMP-2 being retained in the collagen vitrigel membranes without release (J. Zhao et al., 2009). Six percent of the total BMP-2 was released on day one from both the normal gel and the vitrigel membranes, while 33% was released from the normal gel and 15% from the vitrigel membranes within 15 days (J. Zhao et al., 2009). The materials of the membranes also influenced GF release kinetics. Michalska et al. reported that collagen membranes exhibited the highest degree of PDGF-

BB release, and that a much lower release was observed from fibrin membranes up to 120 hours (Michalska et al., 2015). Similarly, approximately 5% of the initially incorporated BMP-2 was released from PLGA membranes within the first 5 minutes, but no substantial release was observed thereafter (Ono et al., 2013).

The release kinetics of different GFs in chitosan-alginate membrane has been also compared, demonstrating that PDGF-AB experienced significantly greater release when compared to TGF-β (Michalska et al., 2008). Moreover, the GF incorporation methods also influenced GF release from the barrier membrane. Park et al. showed that membranes with covalently immobilized rhBMP-2 retained more than 50% of the active BMP-2 for up to four weeks, whereas membranes with adsorbed BMP-2 lost nearly 90% of the initial growth factor within four weeks (Park et al., 2006).

Lee et al. investigated the effect of the concentration of GFs on PDGF-BB release from PLA-TCP membranes (S. J. Lee et al., 2001). Specifically, PDGF-BB was released at a rate of 1.5, 3.2, 5 and 11 ng per day from 60, 125, 250 and 500 ng loaded PLLA-TCP membranes, respectively (S. J. Lee et al., 2001).

Park observed similar trends in release rates and loading behavior (Park et al., 1998). Furthermore, they showed the release of PDGF-BB to be enhanced as the BSA content increased. The release of 100 ng PDGF-BB loaded membrane containing BSA (10%) was almost the same as that from 200 ng PDGF-BB loaded membrane without BSA (Park et al., 1998). Heparinization of the membranes also affects the GF release properties. In this regard, 78.24±10.6% of FGF-2 was released within 24 hours from PCL/gelatin fiber meshes where FGF-2 was physically adsorbed without heparinization - indicating an initial burst release. When FGF-2 was adsorbed with heparinization, the burst release of the GF was up to 17.37±3.3% within 24 hours indication a much lesser burst release (J. H. Lee et al., 2015).

Only Takita et al. examined *in vivo* release kinetics of GFs from membranes. Iodine 125 (^{125}I)-labeled BMP-2 measurements in the dorsal subcutaneous tissue of mice showed BMP-2 to be retained in FGM for more than 14 days (Takita et al., 2004). Half of the rhBMP-2 was loaded in FGM and persisted until 10 days after *in vivo* implantation (Takita et al., 2004). The release properties of GFs from the membranes depended upon the membrane materials, additional reagents such as heparin, cross-linkers, different GFs, and GF concentration.

4. Discussion

Tissue engineering membranes with controlled long-term release of GFs are constructed in an attempt to mimic the extracellular matrix to release endogenous growth factors (Wu et al., 2011). The scaffolds for tissue engineering should exhibit biocompatibility with the tissues where they will be implanted, biodegradability, adequate mechanical properties, and sufficient porosity to facilitate adsorption and diffusion of GFs and cell migration (O'brien, 2011). These ideal properties of scaffolds match the properties of ideal membranes for GBR including the ability to be functionalized by GFs (Caballe-Serrano et al., 2018). The present study has reviewed the GF adsorption and release kinetics from GTR/GBR membranes.

Growth factors have short half-lives, and it is impossible for exogenous GFs to play their regenerative role without the aid of a carrier to control their release. The ideal carrier should be able to have a constant degradation corresponding to the tissue regeneration accompanied by a sustained release of the GF maintaining a therapeutic concentration of the GF (Vo, Kasper, & Mikos, 2012). Sustained delivery of GF such as BMPs into the bone defect site is advantageous for long-term bone regeneration compared with a single high-dose burst of BMPs (Winn, Uludag, & Hollinger, 1998; Woo et al., 2001). Growth factor release is characterized by two phases. In the first phase, burst release from the membranes is observed especially during the first 24 hours. This process comprises various changes in GF concentration of the surface layer, where particle release is easier. The second phase in turn corresponds to the effective delayed release of GFs from the deeper layers of the membrane (Kubis, Musial, & Szczesniak, 2002; Michalska et al., 2015). It seems to be clear that a continuous release of growth factors mimics better the biology of regeneration.

A wide amount of biomaterials can be used as drug carriers for regeneration. Organic and inorganic carriers differ in the loading method, where inorganic components are loaded by a simple adhesion whereas organic carriers provide a higher variability respect to degradation, chemical modifications and growth factors bonding (Schliephake, 2010). Collagen has been found to have infinite treatment options in the field of regenerative medicine, where it can be used as a protein drug carrier (Friess, 1998). Collagen-based membranes have been a popular choice because of their excellent bioactivity, biocompatibility, biodegradable potential and mechanical properties (Marco C Bottino et al., 2012; Caballe-Serrano et al., 2018; Ho et al., 2017). Collagen-based membranes afford excellent GF resorption, retention and release. Chitosan membranes also have gained interest in the field of regeneration due to their flexibility, biocompatibility, biodegradability, antibacterial properties and low cost (Teng, Lee, Wang, Shin, & Kim, 2008; Xu, Lei, Meng, Wang, & Song, 2012). A controlled release of growth factors is of prior importance to maximize any bone regeneration, where collagen and its modifications have proven to increase the release kinetics of growth factors (Vo et al., 2012). Moreover, it is easy to process chitosan into membranes, gels, nanofibers, beads, nanoparticles, scaffolds and sponge forms (Xu et al., 2012). Four studies investigated chitosan-based composite membranes. Lee et al. compared the

adsorption potential of chitosan and chitosan–silica xerogel hybrid membranes, and found the amount of BMP-2 adsorbed onto pure chitosan membranes to be smaller than onto the hybrid membranes (E. J. Lee & Kim, 2016). Furthermore, various polymers such as PCL, poly(DL-lactic acid), poly(lactic-co-glycolic acid) (PLGA), and PLA have been widely used for bone tissue engineering. When manufacturing the membranes by mixing GFs in polymers, the membranes release GF more slowly, with high retention in the membrane for long periods of time. Ono et al. showed PLGA membranes to retain 94% of the initially applied BMP-2 (Ono et al., 2013).

The procedures used to incorporate GFs to the membranes also affect GF release. The immobilization of BMP-2 on polymers through chemical conjugation showed much greater immobilization efficiency and a more controlled kinetic release than those processed through physical adsorption (Kim et al., 2012). Physical adsorption using soluble GF may not be enough to promote long-term implantation, because of drawbacks including protein desorption and/or exchange in contact with physiological fluids (Kim et al., 2012). In contrast, chemical binding involves covalent attachment of the target molecule to the solid surface, resulting in irreversible binding with high levels of surface coverage that makes this approach more suitable. The immobilization of rhBMP-2 on GBR nanofibrous membranes was reported to afford marked osteoblast activity primarily around the membrane, which was be applied for *in vivo* bone regeneration purposes (Park et al., 2006)

Other aspects also influence the release of GFs. Sadeghi et al. showed that the incorporation of fibronectin (FN) to the membranes slowed the release of IGF-I into the medium and enhanced the migration of human gingival fibroblasts in the collagen gels (Sadeghi et al., 2018). Moreover, heparin-functionalized scaffolds are known to enhance sustained release of growth factors and limit the loss of bioactivity (Wu et al., 2011). Sun et al. showed the *in vitro* release curve of P28 peptide to be characterized by initial release from heparin-functionalized mSIS in a small burst, followed by a slow and sustained release maintained over time due to improvement of the efficacy of peptide immobilization on the membrane by heparin (T. Sun et al., 2018). Lee et al. also confirmed that heparinized membranes showed slightly greater FGF-2 immobilization yield than non-heparinized membranes (J. H. Lee et al., 2015). The degree of heparinization might act as a limiting factor in the incorporation of GF to the fibers (J. H. Lee et al., 2015).

The releasing kinetics of GFs were tested using *in vitro* ELISA, high-performance liquid chromatography (HPLC), confocal laser scanning microscopy (CLSM) or radioactive GF measurement assays. However, it was necessary to investigate the bioactivity of the released GFs, since deactivation could possibly occur following release. Most studies investigated GF release using *in vitro* cell assays and/or *in vivo* animal models. In order to confirm the efficacy of the membrane as a GF delivery carrier, the cytoactivity of the GFs released from the membranes was evaluated. For osseoinductive GFs/peptides such as BMP-2, -9 and P28, release was tested based on osteogenic marker expressions including collagen, osteocalcin (OCN), osteopontin (OPN) and alkaline phosphatase (ALP) in mouse bone marrow stromal cells

(mBMSCs)(Ono et al., 2013), murine C2C12 skeletal myoblasts (Caridade et al., 2015), mouse bone stromal cell-line ST2 (Fujioka-Kobayashi et al., 2017), mouse preosteoblasts MC3T3-E1 (Kim et al., 2012; E. J. Lee & Kim, 2016; Park et al., 2006; J. Zhao et al., 2009), rat bone marrow stromal stem cells from ovariectomized rats (rBMSCs-OVX) (T. Sun et al., 2018), rat bone marrow-derived mesenchymal stem cells (rBMMSCs) (Yin et al., 2017), human mesenchymal stem cells (hMSC) (E. J. Chung et al., 2013), human bone marrow mesenchymal stem cells (BMMSCs)(Zhu et al., 2013), and MG63 osteoblast-like cells (Milella et al., 2001). *In vivo* chitosan-based membranes were evaluated by a calvarial defect (5 mm in diameter) model in rats; the rates of defect closure and bony tissue formation were assessed after two weeks of implantation (E. J. Lee & Kim, 2016). The results obtained indicated that the hybrid membrane treated with BMP-2 induced more effective bone regeneration, with a defect closure of 79% (E. J. Lee & Kim, 2016).

The present systematic review has revealed that the adsorption and release kinetics vary among the different materials, forms and GFs. Future studies should focus on the standardization of adsorbtion/release abilities of carriers. The present literature lacks of a clear standardization and conclusions need to be done with caution and limits the present review. It could be proposed, for example, to use normalization based on ng of GF per mass per hour to standardize the adsorbtion/release of GFs on carriers. Despite the limitations of the present review, it can be concluded that membranes can be used as carriers for GFs. Nevertheless, the ability of adsorbtion/release of GFs will greatly depend on the membrane material and manufacturing method.

No.	Authors	year	Tested membrane	Membrane size	Incorporated GFs/molecules	GF concentration/dose	Incorporation method	Membrane adsorption potential	Tested releasing GF	GF release assay method	Tested release time	Release kinetics
1	Sadeghi R	2018	COL-Vicryl® membrane	8 mm diameter	IGF-I and/or FN	IGF-I (100 ng/ml), FN (10 µg/ml)	IGF-I / FN + Vicryl meshes were inserted into the COL and incubated at 37°C in 5% CO2 for 2 hours	COL+FN; 1.5 ± 0.1 ng/ml, COL; 1.4 ± 0.1 ng/ml	IGF-I	ELISA	Up to 14 days	The incorporation of FN to the collagen+Vicryl membranes retained IGF-I in the membranes
2	Imamura K	2018	Cross-linked bovine type L collagen (Biomete 3I, Palm beach	6 mm diameter	FGF-18	4, 7 and 10 ng/ml	Incubated at room temperature for 1 h	-	FGF-18	ELISA	Up to 21 days	A sustained release of FGF-18 from the CM was observed over 21

			gardens, FL, USA)								days
3	Sun T	2018	mSIS membrane derived from porcine jejunum	5×5×0.5 mm3	P28 (BMP-2-related peptide)	3 mg	Heparinized or pure mSIS was incubated with 1 ml aqueous BSA solution (5%) containing P28 for 24 h at 37°C in a humidified atmosphere	Heparinized mSIS; 86.32%±4.27 %, pure mSIS; 62.15%±3.76 %	P28	HPLC	Up to 37 days	The heparin-functionalized mSIS showed a more controlled release process as compared with pure mSIS
4	Ho MH	2017	FGM (collagen core layer+MTZ on outer surface)	-	PDGF	0,30 %	The nanofibers encapsulating 3% MTZ and PDGF in PDLLA were electrospun and deposited on	75.46%±23.14%	PDGF	HPLC	Up to 28 days	PDGF showed sustained-release profiles from the nanofibrous layers over a

							the surfaces of the core layer collagen membrane					period of 28 days with insignificant initial burst release in the first 24 hours
5	Fujioka-Kobayashi M	2017	PPCM (Jason® membrane), PDCM (Mucoderm®)	-	BMP-2, BMP-9	100 ng/ml	Each collagen membrane was placed at the bottom of 24-well plates and coated with BMP-2 or BMP-9 in DMEM for 5 minutes	Approximately 90%	BMP-2, BMP-9	ELISA	Up to 10 days	Both BMP-2 and -9 adsorption onto PPCM and PDCM showed slow BMP release over time for up to 10 days overall
6	Yin L	201	Core-shell	100 mg	BMP-2,	BMP-2 (10	Shell fluid	2.213 - 3.225	BMP-2,	ELISA	Up to	BMP-2 and

		7	SF/PLLACL fibrous membrane (mass ratio 30:70)		IGF-2	µg/ml), IGF-1 (10 µg/ml)	(8% SF/PLLACL in hexafluoroisopropanol) and core fluid (BMP-2 or IGF-1 in PBS) were combined by the coaxial electrospinning device at a temperature of 22–25°C and with a relative humidity of 40–60%	µg	IGF-2		28 days	IGF-1 were released gradually and were sustained until 28 days, with maximum releases of > 60%
7	Edelmayer M	2017	Collagen membrane (BioGide®)	5 mm diameter	DFO, DMOG	3 mM	50 ml of the PHD inhibitors solution	-	DFO, DMOG	HPLC	Up to 2 days	Most of DFO and DMOG were

							were applied to the membrane at room temperature. The samples were frozen at -80ºC and lyophilized with a freeze dryer					released within the first hours
8	Mozgan EM	2017	Collagen membrane (BioGide®)	5 mm diameter	Washed PSEC, unwashed PSEC	1 x 109 platelets/ml	Soaked with PSEC, frozen at -80°C and lyophilized with a freeze dryer	-	PDGF-BB, TGF-β1	ELISA	Up to 2 days	Most growth factors was released within the first 6 h. Unwashed PSEC-loaded CBM released more

												protein, PDGF-BB, and TGFβ1 than washed PSEC-loaded CBM
9	Takayama T	2017	Collagen membrane (OsseoGuard®)	7 mm diameter (200 mm thickness)	SDF-1	5 ng	SDF-1a solution was dropped onto the membranes and incubated at room temperature for 1 h	-	SDF-1	ELISA	Up to 21 days	Approximately 10% of sustained SDF-1 release from the CM was observed for 3 weeks
10	Du M	2017	ADM	10 × 8 × 1 mm	FGF-2, BMP-2	FGF-2 (200 ng/ml), BMP-2 (800 ng/ml)	Coating 0.5 ml of FGF-2 or BMP-2 on membranes,	-	FGF-2, BMP-2	ELISA	Up to 14 days	FGF-2 and BMP-2 have a similar

							incubated at 4°C overnight and then freeze-dried at −60°C					release tendency over time, with a release of 80% of the total dosages during the first 200 h, followed by lesser drug release.
11	Lee EJ	2016	Chitosan and chitosan–silica xerogel hybrid membranes	10 × 10 mm	BMP-2	20 μg/ml	The membranes were immersed in PBS solution containing BMP-2 and then incubated for 3 h at	The amount of BMP-2 adsorbed on the pure chitosan membrane was less than that adsorbed on the hybrid	BMP-2	BMP-GFP, CLSM	Up to 20 days	BMP-2 was released steadily from both membranes for an extended period of time. After 20 days,

							37°C	membrane				the total amounts of BMP-2 released from the hybrid and pure chitosan membranes were approximately 3.5 and 2 µg, respectively
12	Michalska M	2015	Fibrin, collagen, fibrin-heparin, fibrin-collagen membrane	4 mm diameter	PDGF-BB	0.25 µg/ml	100 µl of PDGF-BB was introduced into membrane polymers under aseptic	-	PDGF-BB	ELISA	Up to 5 days	The collagen membranes showed the highest level of PDGF-BB release,

							conditions and evaporated.					while much lower release was observed from fibrin membranes
13	Lee JH	2015	PCL/gelatin composite fiber meshes	10×10 mm	FGF-2	50 or 100 ng/ml	The heparinized fibrous meshes were immersed in 500 μl of FGF-2 solutions for 24 h at room temperature	100 ng/ml of FGF-2; 21.95±4.5% heparinization+50 ng/ml of FGF-2; 58.60±2.5% heparinization+100 ng/ml of FGF-2; 29.25±7.6%	FGF-2	ELISA	24 hours	78.24±10.6 % of FGF-2 was released within 24 h from the PCL/gelatin fiber meshes, indicating initial burst release. The percentages of FGF-2

												released from heparinized membranes 50 ng/ml, 100 ng/ml were 3.69±1.0 and 17.37±3.3 %, respectively, indicating that burst release was significantly reduced
14	Caridade SG	2015	Chitosan/alginate FS membrane	~ 1 cm2	BMP-2	20 µg/ml, 60 µg/ml or 100 µg/ml	Membranes were immersed in 1 mM HCl solution (pH	The loaded amounts depended on the initial concentratio	BMP-2	CLSM	Up to 1 month	Increased BMP-2 loading when the initial BMP-

							= 3) for about 1 h. After removal of the HCl solution from the wells, the membranes were incubated with the BMP-2 solution (overnight at 4°C)	n of BMP-2 in solution and the degree of crosslinking of the FS membrane				2 concentration is increased and increased percentage release for the less crosslinked film/membrane. After an initial burst, the growth factor was released over one month through diffusion
15	Shim JH	2014	PCL/PLGA/β-	10 mm diameter	BMP-2	50 ng	PCL/PLGA/β-TCP fibers	-	BMP-2	ELISA	Up to 28	25.5% of total

			TCP membrane				and collagen/rhBMP-2 solution were separately dispensed into each layer by a 3D printer				days	rhBMP-2 was released within 24 h. After this initial burst release, sustained release was observed for up to 28 days. Up to 47.2% of total rhBMP-2 was released by day 28
16	Hamido	2015	Collagen membrane (BioGide®)	5 mm diameter	DMOG, L-MIM	3 mM	50 ml of the prolylhydroxylase inhibitors solution was	-	DMOG, L-MIM	Direct measurement by a DU530	Up to 48 hours	Most DMOG and L-MIM was released within the

							added to the membrane at room temperature and lyophilized with a freeze dryer			life science UV/Vis spectro photom eter, or colorim etric assay		first hours, with the highest levels in the first hour
17	Ono M	2013	PLGA membrane	-	BMP-2	1 µg/µl	The membranes were incubated with 1 µg/µl of BMP-2 solution at 4°C for 24 h	-	BMP-2	ELISA	Up to 180 min	PLGA membrane retained 94% of the initially applied BMP-2
18	Chung EJ	2013	Self-assembling collagen-HyA membranes	-	BMP-2	5.71 µg/ml	2 µg of human recombinant BMP-2 was added to 350 µl of collagen	-	BMP-2	ELISA	Up to 49 days	17% of the total BMP-2 incorporated was released by

							solution before overlaying onto 350 µl of HyA solution and incubated at room temperature for 24 h					day 49
19	Zhu H	2013	PEG/PCL membrane	10 × 10 mm	BMP-2	0.5 µg	Coaxial electrospinning was conducted over 2 hours. PCL solution was used to form the outer shell and the PEG/BMP-2 solution was used to form	-	BMP-2	ELISA	Up to 24 days	Approximately 500 pg of BMP-2 was released from the membrane per day

							the inner core					
20	Nguyen TH	2013	Micro- and macro-porous fibrous scaffold made of blended polystyrene (PS) and PCL	-	BMP-2	1 µg/ml	Immersion in the prepared rhBMP2 solution and incubated overnight at 4ºC	-	BMP-2	ELISA	Up to 8 weeks	Sustained GF release was observed up to the first week, after which release started to decrease nonsignificantly
21	Kim JE	2012	PDO membrane	0.5 × 0.5 cm2	BMP2	20 µg/ml	BMP-2 solution was treated on the membrane for 10 days	-	BMP-2	ELISA	Up to 21 days	Immobilization of BMP-2 on PDO membrane had a retention efficiency of 8.6 ng in 7 days.

												After 21 days, 0.4 ng/ml of the loaded BMP-2 had been released from PDO membrane
22	Hong KS	2010	Hybrid membrane consisting of collagen and nBG	8 mm diameter	FGF-2	10, 50, and 100 μg/ml	100 μl of FGF2 solution (in α-MEM) was dropped onto the membrane and left to stand for 2 h at room temperature	The FGF2 adsorbed onto the membrane was estimated to be 4.68, 39.5 and 57.3 μg/ml with the initial treatment of 10, 50 and 100 μg/ml, respectively	-	-	-	-

23	Zhao J	2009	Type I collagen normal gel, collagen vitrigel membranes	35 mm diameter	BMP2	60 µg/ml	Equal volumes of 0.5% acid-solubilized type I collagen solution and BMP-2 solution were mixed and gelatinized, vitrified and rehydrated	-	BMP-2	ELISA	Up to 15 days	The release rate showed a burst on day 1 in both types of gels. About 33% of the total BMP was released from the normal collagen gel and 15% of the total BMP was released from vitrigel within 15 days
2	Michal	200	Chitosan-	10 × 10	PDGF-AB	10 µg	During	-	PDGF-	ELISA	Up to	PDGF-AB

4	ska M	8	alginate membrane	mm	and TGF-β	PDGF-AB in 1.11 ml PBS and 5 µg TGF-β in 3.921 ml PBS	mixing of the polymer ingredients, GFs were introduced and evaporated		AB and TGF -β		5 hours	was released faster from the membrane than TGF-β.
25	Park YJ	2006	Chitosan nanofibrous membrane	-	BMP2	2 or 5 µg	BMP-2 in PBS was incubated with the membrane for 10 h at 4ºC	The amount of immobilized BMP-2 increased in accordance with the content of the cross-linker SMCC.	BMP-2	Radioactive BMP-2 measurement	Up to 4 weeks	The membranes with covalently immobilized rhBMP-2 retained more than 50% of the active GF for up to 4 weeks, whereas membranes with adsorbed

												rhBMP-2 lost nearly 90% of the initial GF within 4 weeks
26	Takita H	2004	Fibrous glass membrane	10 × 5 × 1 mm	BMP2	8.7 µg	The membranes (6 mg) were mixed with BMP-2, lyophilized and stored at -80°C until use	-	BMP-2	Radioactive BMP-2 measurement	Up to 14 days	Half of the amount of rhBMP-2 as loaded in the membranes remained until 10 days after *in vivo* implantation
27	Lee YM	2003	PLLA/TCP membrane	Height 4 mm, diameter 8 mm, thickness	BMP2	5 µg	At least 30 minutes after BMP-2 solution had soaked into	-	BMP-2	ELISA	Up to 4 weeks	70% of BMP-2 was released during the first day.

				0.5 mm			the membrane, the latter was freeze-dried and kept at -20°C until required					Thereafter, BMP-2 was consistently released at a rate of 7-10 ng/day
28	Milella E	2001	PLLA–alginate membrane	15 mm diameter	TGF-β	20 ng/ml	TGF-b solution (PBS containing 0.2% BSA) was added to the cross-linked alginate membranes and incubated for 4 h at 37°C	-	TGF-β	ELISA	Up to 7 days	Similar release kinetics with a high amount of GF delivered on the first day, followed by constant release
29	Lee SJ	2001	PLLA/TCP membrane	Height 3 mm,	PDGF-BB	60, 125, 250 and	PDGF-BB was incorporated	-	PDGF-BB	Radioactive	Up to 14	A therapeutic

				diameter 8 mm, thickness 150 µm		500 ng	to the PLLA solution and TCP was added to PLLA in 50:50 (w/w) ratio to polymer weight. PLLA-TCP solutions were cast on a dome-shaped metallic mold and evaporated			BMP-2 measurement	days	concentration range of PDGF-BB was continuously released from the PLLA-TCP membranes
30	Park YJ	1998	PLLA porous membrane	10 × 10 mm	PDGF-BB	100, 200, 400 ng	Coating PDGF-BB-dissolved PLLA methylene chloride–ethyl acetate	-	PDGF-BB	Radioactive BMP-2 measurement	Up to 4 weeks	The release of PDGF-BB was enhanced as the BSA content increased.

							solutions on PGA meshes					The release rate increased proportionally as the loading GF content increased
31	Chung CP	1997	PLLA porous membrane	1 cm2	PDGF-BB	-	Coating PDGF-BB-dissolved PLLA methylene chloride–ethyl acetate solutions on PGA meshes	-	PDGF-BB	Radioactive BMP-2 measurement	Up to 7 days	PDGF-BB was slowly released from uncoated membrane. After 1 day, both coated and uncoated membranes showed a similar constant release.

												The release of PDGF-BB was enhanced by the coated PVA membrane

COL; collagen, IGF; insulin-like growth factor, FN; fibronectin, BSA; bovine serum albumin, mSIS; mineralized decellularized matrix from the small intestinal submucosa, HPLC; high-performance liquid chromatography, FGM; functionally graded membrane, MTZ; metronidazole, PDLLA; poly(L-lactide-co-D/L-lactide), PPCM; porcine pericardium collagen membranes, PDCM; porcine dermis-derived collagen membranes, SF; silk fibroin, PLLACL; poly (L-lactide-co-caprolactone), DFO; deferoxamine, DMOG; dimethyloxalylglycine, PHD; prolylhydroxylase, PSEC; secretome of platelets, SDF-1; stromal cell-derived factor-1, ADM; acellular dermal matrix, CLSM; confocal laser scanning microscopy, PBS; phosphate buffer saline, PCL; polycaprolactone, FS; free-standing, PCL; polycaprolactone, PLGA; poly(lactic-coglycolic, β-TCP; beta-tricalcium phosphate, L-MIM; L-mimosine, PLGA; polylactide-co-glycolide, HyA; hyaluronic acid, PCL; poly(caprolactone), PEG; poly(ethylene glycol), PDO; poly(dioxanone), nBG; nano-bioactive glass, α-MEM; α-minimal essential medium, vitrigel; a stable collagen gel membrane prepared from vitrified type I collagen, SMCC; [succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate], PLLA; poly(L-lactide), TCP; tricalcium phosphate, PGA; polyglycolic acid. Soaking; soaking membranes in GF solution and incubation, Lyophilization; soaking with PSEC, frozen at -80 °C and lyophilized with a freeze dryer, During membrane fabrication; incorporation of GFs during membrane manufacture

6. Competing interests

The authors declare that they have no competing interests.

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8. Author contributions

J.C.-S., R.M. and F. H.-A. designed the search protocol and the study. Y.A.-M. and M. F.-K. contributed to the literature research with the help of J.C.-S. and A. M.-F. The manuscript was written by J.C.-S., Y.A.-M., M.F.-K. and A.M.-F. All authors reviewed, edited and approved the final manuscript.

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