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# The Effect and the Potential Use of Magnetic–Dam Barrier in Guided Bone Regeneration: A Laboratory Study

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**Abstract:** Guided bone regeneration (GBR) has been shown to be an optimal technique to accelerate the bone regeneration process thanks to the action of membrane barriers that promote tissue healing through the process of osteogenesis, inducing the repopulation with osteoprogenitor cells that prevent the invasion of non-osteogenic tissue. However, current membranes, such as expanded polytetrafluoroethylene or rubber dam, have some disadvantages that could potentially reduce the effectiveness of GBR. Recently, some scaffolds with magnetic properties have been tested to promote rapid osteogenesis. The aim of this laboratory study was to evaluate the intensity of the magnetic field generated by a custom-made rubber dam magnetised with neodymium-iron-boron (Nd2F14B) (three layers of latex filled with Nd2F14B powder on the inner surface) and to understand the effects of such a membrane on cell viability. A magnetic field of 750 G, 400 G, and 900 G was generated on the surface and on the long and wide sides of 3 and 2 cm in contact with the rubber dam. At a distance of 1 mm from the magnetic dam, a magnetic field of 300 G, 150 G, and 400 G was measured on the surface and on the long and wide sides of the rubber dam, respectively. After 72 h, the MG-63 osteoblast-like line showed a slight decrease in cell proliferation (85 $\pm$ 10) compared with the unmodified dam (95 $\pm$ 6) and the cell control population. According to our findings, this magnetic cofferdam is able to generate a static magnetic field and significantly affect cell proliferation in contrast to other nonabsorbable membranes. Further laboratory studies and subsequent clinical trials are needed to evaluate the significant improvements that can be achieved by using this type of magnetic rubber dam in GBR.

**Keywords:** guided bone regeneration; magnetic dam; membrane barrier; neodymium-iron-boron; static magnetic fields



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## 1. Introduction

Guided bone regeneration (GBR) is a surgical technique that allows the regeneration of bone at anatomical sites with vertical, horizontal, or combined defects or atrophies. Its efficacy is based on the ability to control new bone formation using a membrane barrier that prevents the settlement and proliferation of mucosal connective tissue in the bone wound and allows the colonisation of the area by totipotent osteoblastic cells from the medullary spaces [1]. It is thought that bone regeneration is achieved when osteoprogenitor cells alone repopulate the bone defect site by preventing the invasion of non-osteogenic tissue. It is estimated that up to 40% of osseointegrated implants require GBR as part of the patient's rehabilitation, and the survival rate of implants placed at augmented sites with GBR varies from 79% to 100%, with a survival rate of over 90% after at least one year of function [2].

Osteogenesis achieved by GBR consists of the same phases that characterise the physiologic process of new bone formation; therefore, between 4 and 6 months are required

for complete healing. During this period, the maintenance of a membrane barrier is necessary for the regeneration process to be completed: the factors related to the extent and morphology of the defect, vascularisation, and quality of the surrounding bone are critical [3].

Nonabsorbable membranes are by far the most common and widely used membranes in GBR. They include expanded polytetrafluoroethylene (e-PTFE), the non-absorbable membrane, which is considered the gold standard in many studies, unexpanded e-PTFE, rubber dam, and titanium membrane or grid [3]. The ideal GBR membrane should be sufficiently stiff to withstand the pressure of the overlying soft tissue, it should also have some degree of plasticity so that it can easily conform to the shape of the defect, and it should have an optimal degree of porosity to allow for the diffusion of fluids, oxygen, nutrients, and bioactive substances for cell growth, which is critical for bone and soft tissue regeneration, without compromising the cell-sealing property of the membrane, as soft tissue cells may migrate through the membrane and displace the defect site and inhibit the invasion and activity of bone-forming cells [2]. Specifically, rubber dam has proven effective in preventing the accumulation of plaque and the passage of bacteria, which is the case with e-PTFE [4], and in accelerating the healing of bone defects in rat calvaria after 15, 30, 60, and 120 days from the application and in achieving a higher degree of mineralisation at 60 and 120 days [5]. Unfortunately, the smooth surface of the rubber dam may present some drawbacks due to the receding of the margins over the latex and the consequent exposure of the membrane, which can produce undesirable aesthetic effects and reduce the probing depth in the newly formed tissue [4].

In recent years, tissue engineering has focused on the development of magnetic materials for bone regeneration [6–9]: numerous scaffolds with magnetic properties have been tested to serve not only as a temporary matrix and to support cell growth and secretion, but also to promote osteogenesis a few days after their application thanks to the action of static magnetic fields (SMF) generated by the combination of hydroxyapatite and γ-Fe3O4 nanoparticles [6], hydroxyapatite and magnetite in the ratios HA /Mgn 95/5, HA /Mgn 90/10, and HA /Mgn 50/50 [7], and $Fe_3O_4$ [8] with good biodegradable abilities [9]. Recently, an innovative type of implant (Cover Screw Supercharged) has been introduced, consisting of a modified neodymium-iron-boron permanent magnet ($Nd_2F_{14}B$) which is placed inside the device with optimal magnetic properties to accelerate the healing times of the bone around the implant and, consequently, the patient's prosthetic rehabilitation phases [10]. In vitro studies have shown that these SMF have no cytotoxic effects on MG-63 osteoblasts, which after 72 h of contact with the implant, show the overexpression of genes that are important for the osteogenic process, such as the genes encoding collagen, tGFβ1, BMP2, ALP, amelogenin, VEGF, and PDGF, thus increasing bone growth in contact with the implant and stimulating endogenous bone repair, thus reducing healing time [10].

Therefore, the aim of this in vitro study is to measure the magnetic field generated by a custom-made rubber dam magnetised with $Nd_2F_{14}B$ and to understand the effects of such a membrane on cell viability.

## 2. Materials and Methods

### *2.1. Construction of the Magnetic Rubber Dam*

A magnetic membrane of size 2 × 3 cm was specially made. The membrane consists of three layers of a hygienic dental dam (Coltene Whaledent) made of natural rubber latex with a thickness of about 0.15 mm, which were fused together by heat. The rubber dam has peculiar physical properties, such as impermeability, which ensures the separation between the outside and the inside of the barrier, elasticity, and which favours its positioning and stability. In addition, the elastic tension of the rubber dam can provide effective support for the tissue so that the space remains well-compartmentalised for bone regeneration [11].

Inside the membrane, there is a neodymium-iron-boron powder (Xiamen Yuxiagn Imp. & Exp. Co., Ltd., Xiamen, China) characterised by an N35 gradation, a maximum operating temperature of 80 °C, a maximum energy product (BH) of 35 MGOe (million

gauss per oersted), and a coercive force (HC) of 860–915 kA/m, while the remanence (Br), i.e., the magnetic induction remaining in the magnet after magnetisation, is 1.17–1.21 T. The area covered by the magnetic dust is about 1.5 × 2.5 cm. The magnetic powder binds to an insulate dam sheet adhered by heat, which is called the base. It is located between two other dam sheets, one of which serves as an inner cover and the other as an outer cover. Thus, the membrane has an inner side that faces the tissue to be regenerated and an outer side that is in contact with the gingival tissue. Thus, on the inner side of the membrane, there is only one layer that is between the magnetic dust and the tissue to be regenerated: the inner cover. On the outside, there are two dam layers between the magnetic dust and the gum tissue: the base in the innermost part and the outer cover in the most superficial part. The topography was not changed by the use of the magnetic powder. Details of the structure of the magnetic dam can be found in the Supplementary Materials.

### *2.2. Experimental Setup*

Three six-wells (I, II, III) were prepared, each characterised by a different incubation time of the cells contained in the wells (I: analysis within 24 h; II: analysis within 48 h; III: analysis within 72 h). The six wells contain:

(1) Cells exposed to the magnetic field (the well contains the magnetic dam);
(2) Cells that are not exposed (the well contains only cells);
(3) Cells in direct contact with the normal dam.

Well 1 represents the test case (exposed), while wells 2 and 3 represent the controls.

#### 2.2.1. Measurement of the Magnetic Field

The presence of a static magnetic field was measured in the wells before the insertion of the cells using a Gaussmeter with a Hall effect probe: Hall-effect transducer—UGN3501U—Allegro Microsystem Inc. The UGN3501U integrated device uses the Hall effect to measure the magnetic field and provides an output voltage that is linearly proportional to the intensity of the sensed flux. The device can also measure slight variations in the magnetic field, which can be applied to an amplifier to achieve higher voltages.

The transducer includes in the same package a hall sensor, linear amplifier, emitter-tracker output, and a voltage regulator.

The operating temperature is between 0 and 70 °C, and the working voltage is between 8 and 10 V.

Specifications of Gaussmeter S.M.F.

- Transducer: Hall effect UGN3501 Allegro Microsystems;
- Conditioning circuit;
- Sensitivity: 1 mV/Gauss;
- Min. and max. range 0–1500 G;
- Error: +/−5% in range +/−1500 mVdc;
- High impedance voltmeter (>10 kOhm);
- Payload +/−1999 mVdc;
- Positive polarity under the specified measurement conditions.

Three series of the SMF field strength measurements were made at the same ambient temperature and under a sterile hood and in contact with the magnetic dam and 1 mm from the surface of the magnetic dam. In each series of measurements, care was taken to control the positioning of the probe with extreme precision.

#### 2.2.2. Cell Cultures

The cells that were used included MG63: osteoblast-like cells from osteosarcoma. The cell line was cultured and maintained as a monolayer on T25cm 2 flasks in a DMEM/Ham's F-12 medium with the addition of 10% fetal bovine serum and 50 μg/mL gentamicin at 37 °C in a humidified atmosphere of 95% $O_2$ and 5% $CO_2$.

For the magnetic membrane experiment, the cell line MG-63 was seeded in plates with six wells, and the following time intervals were chosen at 24 h, 48 h, and 72 h.

The workflow was as follows:

1. The culture medium was removed from the flask and washed with 5 mL of D-PBS (Dulbecco buffered phosphate salt solution) without calcium and magnesium.
2. After removing the D-PBS, 1 mL of 0.05% prewarmed trypsin-EDTA was added.
3. After 5–10 min at room temperature, the cells broke down.
4. After the addition of 5 mL of growth medium containing trypsin inhibitor, the suspension containing the cells was transferred to a centrifuge tube.
5. The sample was then centrifuged at 1200 rpm for 6 min.
6. After the supernatant was aspirated, the cell pellets were re-suspended in the culture medium.
7. The cells were counted and seeded into the six-well plates.
8. For the 24-h timeline, 600,000 cells were seeded into each well; in the 48-h timeline, 300,000 cells were seeded into each well. Finally, for the 72-h session, 90,000 cells were seeded into each well.
9. After seeding the cells, the magnetic dam was placed in the first well, the corresponding control with normal dam was placed in the second well, the cell suspension was placed in the third well, and this mode was performed in the six wells for the periods of 24, 48, and 72 h.
10. The plates with six wells were incubated for 24 h, 48 h, and 72 h in an incubator at 37 °C in an atmosphere humidified with 95% $O_2$ and 5% $CO_2$.
11. Cell growth was assessed after 24, 48, and 72 h by counting the cells in the monolayer.
12. After counting, the cells were centrifuged in D-PBS at 1200 rpm for 6 min.
13. At the end of centrifugation, the supernatant was aspirated, and the cell pellet was frozen at −80 °C.

#### 2.2.3. Analysis of Cell Viability by MTT Test

The MTT test (3-dimethylthiazole-2,5-diphenyltetrazolium bromide) is a colorimetric test used to estimate the number of cells with mitochondrial activity and, thus, cell viability. This assay is based on a metabolic indicator; the soluble salt tetrazolium is reduced in the mitochondrion of viable cells by active dehydrogenase enzymes to a blue-purple crystal that is insoluble in water (formazan). The dissolved crystals are quantified by a colorimetric method at a wavelength of 570 nm (dye absorption) with background correction at 690 nm. At various time points, the culture medium was removed, and 200 µL of a solution containing MTT (Merck, Darmstadt, Germany) (5 mg/mL in medium without phenol red) and 1.8 mL of the medium were added to the monolayer cells. The plates were then incubated at 37 °C for 4 h. After the removal of the supernatant, the blue-violet formazan crystals were dissolved by adding 2 mL of solvent (4% HCl in isopropanol) and were quantified at 570 nm and 690 nm using a spectrophotometer (Secoman, Anthelie light, version 3.8, Contardi, Italy). The results are expressed as a percentage of viability compared to the control cultures. The procedure followed that used by Memè et al. (2022) [12].

### *2.3. Statistical Analysis*

Data analysis was performed using GraphPad Prism software version 8.00 for Windows (GraphPad Software, version 8.00, San Diego, CA, USA). Differences between the groups, based on cell viability assays, were determined using Kruskal–Wallis followed by Dunn's procedure. A $p$-value < 0.05 was considered statistically significant.

## 3. Results

### *3.1. Control of the Generation of the Static Magnetic Field (SMF)*

When measuring the magnetic field with the Hall effect transducer—UGN3501U—Allegro Microsystem Inc., magnetic fields in contact with the dam at 750 G on the surface, 400 G on the sides of 3 cm, and 900 G on the sides of 2 cm were detected. One millimetre

from the magnetic dam, fields of 300 G were measured on the surface, 150 G on the sides of 3 cm, and 400 G on the sides of 2 cm (Figure 1). The direction of the magnetic field measured with the Hall probe was north–south, with the membrane placed on a horizontal surface.

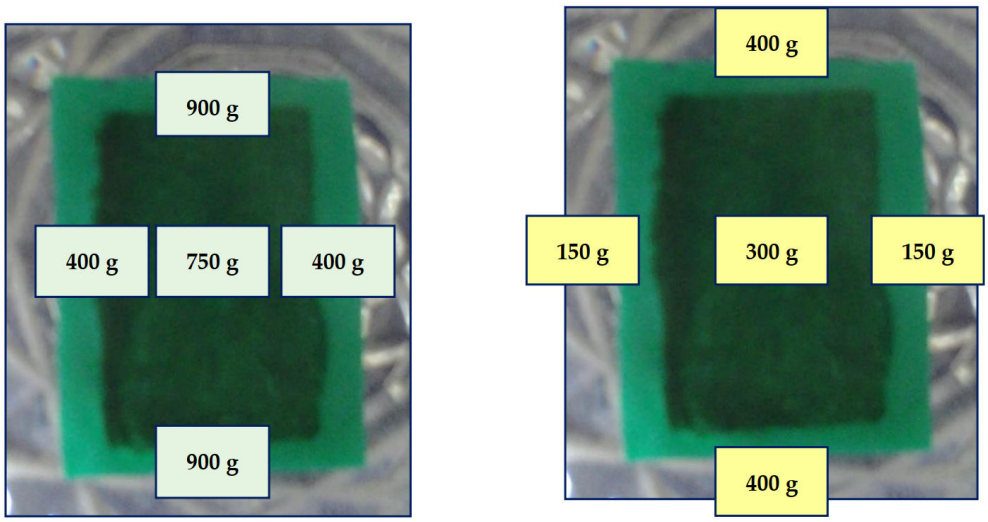


**Figure 1.** Magnetic field (**a**) On contact with the rubber dam; (**b**) At a distance of 1 mm. The different intensities detected at contact with the dam and at a distance of 1 mm were due to the rapid decays with the distance due to its dipolar nature. Despite its small size (2 × 3 cm) and minimal thickness (approx. 0.5 mm), the dam generated a magnetic field whose lines extend into the surrounding area.

### *3.2. Cell Proliferation (MTT Test)*

Cell viability was performed after 24 h of contact between the MG63 cell population and the magnetised dam and showed no statistically significant differences compared with the controls (normal dam and cells only). After 72 h of culture, the MG-63 osteoblast-like line showed a slight decrease in cell proliferation (85 ± 10) compared with both the unmodified dam (95 ± 6) and the cell control population. The complete results are reported in Figure 2.

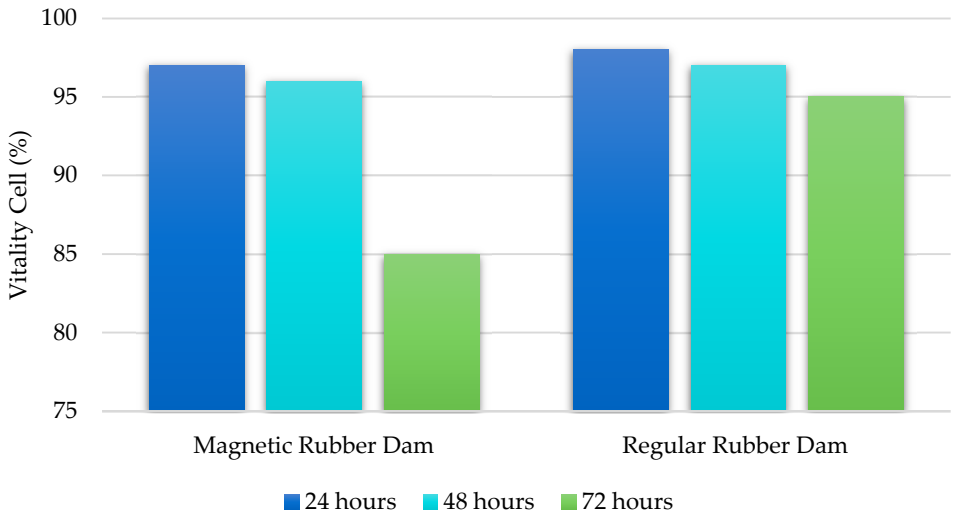


**Figure 2.** Percentage of vitality cell determined related to the control group.

## 4. Discussion

In dentistry, the stimulation with a static magnetic field (SMF) produced by permanent magnets is commonly used to accelerate tooth movements induced by orthodontic appliances [13,14]. The application of orthodontic force in combination with an SMF allows the acceleration of root resorption, periodontal ligament space enlargement, and TRAP activity (resistant acid tartrate phosphatase, marker of osteoclastic activity) after 7 days of application compared to orthodontic force alone [15]. SMFs play an important role in promoting mandibular growth in class [16] malocclusions and in the conventional retention of overdentures or implants [17] because they have no adverse effects on human gingival fibroblast enzymes [18]. SMFs are, therefore, non-invasive procedures that are recommended for patients with a reduced number of teeth and poor alveolar support who require prosthetic rehabilitation.

Our study aims to investigate how the use of a cofferdam can be applied as a non-absorbable membrane for GBR, which can generate a static magnetic field thanks to an $Nd_2F_{14}B$ powder contained in it and affects cell proliferation. Unlike other membranes for GBR, such as electrically charged e-PTFE, which should be negatively charged to accelerate bone formation [19], the time-invariant static magnetic field (SMF) is generated by permanent magnets or electromagnets in which a constant direct current (continuous current) flows does not require any special properties or equipment to be easily used, and this makes this stimulation method suitable for long-term local treatments, such as dental treatments [20]. In general, with the exception of ferrite magnets, the durability of magnets is unlimited if overheating processes of the magnet at temperatures close to the Curie point of the materials they are made of (always above 300 °C) is avoided. Recent studies have demonstrated the existence of numerous receptors in biological systems for weak SMF in combination with alternating magnetic fields. Indeed, these stimulate the production of tumour necrosis factor (TNF) by macrophages, decrease chromatin resistance to DNAase-1, and increase protein hydrolysis both in vivo and in vitro [21]. According to a study by Abdi et al. (2013), static magnetic fields may be involved in the pathogenesis of atherosclerosis because they can alter LDL metabolism and the interaction of these with other molecules [22]. However, SMFs are not a therapeutic variant of EMFs in clinical practice [20,23]. According to some in vivo studies of stimulation with permanent magnets, SMFs have a clear effect on osteogenesis, but the mechanisms of action on osteoblasts and bone metabolism are unclear [20,23]. The physical response of the tissue is undoubtedly different from that induced by exposure to PEMFs. SMFs do not generate an electric field in the tissue, only a magnetic field [24–26], since it is not a stimulation that provides energy in any form but is a quantity of state similar to the gravitational field [27]. In this way, SMFs do not produce electric currents or vectorial changes but a biologically derived EMF with a cascade of intracellular signaling pathways [28] that directly promote the differentiation of osteoblasts and bone maturation [29]. Since the intensity of static magnetic fields depends on the mass of the permanent magnets that generate them, the achievable intensity is particularly low. However, in vitro and animal studies show that this intensity leads to an increase in the energy differentiation of cells (osteoblasts) and does not induce cell replication, making static magnetic fields safe for soft tissue [30].

From our work, we observed that during the fabrication of the magnetic dam, it was mainly observed that the adhesion of the magnetic neodymium-iron-boron powder to the latex dam did not change the properties of the dam, but the latex sheets that were bonded by heat could maintain the adhesive properties of the latex even in the cooling phase. The specially designed magnetic membrane could enable its clinical application by combining the handling, adaptability, cost-effectiveness, and excellent barrier properties of the cofferdam 51–56 with the effect of static magnetic fields on the bone tissue. Magnetic fields of 750 G at the surface, 400 G at the 3 cm sides, and 900 G at the 2 cm sides in contact with the cofferdam were detected when the magnetic field was measured using the Hall-effect transducer—UGN3501U—from Allegro Microsystem Inc. Fields of 300 G were measured one millimetre from the magnetic dam at the surface, 150 G at the sides of 3 cm, and 400 G

at the sides of 2 cm. This shows that despite its small size and thickness (about 0.5 mm), the dam is capable of generating magnetic fields that can affect the surrounding tissue.

The results of the in vitro experiments show the same inconsistency of results found in the current literature. According to many studies, the stimulation with SMF seems to have a negative effect on proliferating cells while leading to an increase in the rate of differentiation, activation, and mineralisation [31–34]. In contrast, other studies show that SMF within a certain range of reduced magnetic intensity, on the order of mT, increases cell growth in a dose-dependent manner [35,36]. However, given the paucity of studies on static magnetic fields and the large variability in experimental conditions, the relationship between field intensity and cellular effect in terms of proliferation or induction is still unclear. According to our results, the observed slight decrease in proliferation could be due to the high values of magnetic fields developed by the dam (400–900 G at contact, 150–400 G at 1 mm distance). Cell viability analysis performed after 24 and 72 h of contact with the magnetised dam showed no statistically significant differences in the growth of the MG63 cell population compared with the controls, demonstrating the non-toxicity of the dam. This evidence is consistent with the results of Shimizu et al. (2004), who found an increase in GNP in the range of 300 and 800 G 128 after 24 h of cell exposure [25]. Therefore, in agreement with in vivo studies, an increase in mineralisation, bone density, and vascularisation can be assumed to be positive.

This study has some limitations, mainly due to its innovative design, which limits the comparability of our results with other studies. In addition, biological changes due to the presence of a magnetic field were not investigated by alkaline phosphatase analysis. Mild or negligible cytotoxicity was found in cells exposed to coated or recycled magnets; although no exposure of magnet grains to cell culture was observed, it cannot be excluded as a microscopical leakage. When the magnets are not in direct contact with gingival fibroblast cells, there was no significant difference in the cell shape or surface structure, even in areas with a high magnetic field density and steep gradient [30].

This laboratory study is one of the first studies on the application of static magnetic fields in GBR. The use of a magnetic membrane would make it possible to shorten the physiological time of bone healing that is required: 4–6 months. Since the goal of this surgical method is often the placement of osseointegrated implants, a magnetic membrane would allow us to shorten the time required for the morphofunctional restoration of one or more tooth elements, thus significantly improving the patient's quality of life.

## 5. Conclusions

Our laboratory results have shown that the application of $Nd_2F_{14}B$ on a normal rubber dam is able to induce an SFM that significantly reduces cell viability 72 h after implantation and, if applied in a clinical setting, could consequently optimise the effectiveness of the rubber dam in bone regeneration. To this end, further laboratory and subsequent clinical studies on the potential cytotoxicity of such magnetic membranes and the mechanism of action of SMF are needed; although the observed effect of SMF is gradually becoming clear, more in-depth studies on its role in the osteogenesis process should be performed.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13031625/s1, File S1: Magnetic rubber dam construction.

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