

IgG DIFFUSION THROUGH BIOENGINEERED MATERIALS FOR PERIPHERAL NERVE REGENERATION

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INTRODUCTION: In successful nerve regeneration, sprouting axons from the proximal nerve stump traverse the injury site, and make new connections with target organs. Axons that fail to reach an appropriate end organ or fail to make a functional synapse will eventually undergo Wallerian degeneration. However, the incidence of recovery is highly variable, and the return of function is never complete. The use of synthetic nerve guidance channels show promises in improving the repair of injured human nerves, and the release of soluble bioactive agents like cytokines, may improve the degree and specificity of neural outgrowth [1]. The fibronectin (FN) guidance material has been already shown to promote axonal growth of sensory and other axons in rat spinal injuries in a way and to an extent not seen with any other non-graft implant to date. Ingrowth of new nerve tissue was dramatic in speed and content [2]. Also hyaluronan (HA) derivative HYAFF-11 (benzyl ester of Hyaluronic Acid) has been tested to be used for the bioartificial nerve guidance first of all because of its well documented biocompatibility and biodegradability and secondly because its particular physical-chemical properties make it processable in various three dimensional forms.

Considering the role of the cytokine TGF- β 1 in scar formation and the potentially obtainable improved results in the repair of central and peripheral nervous system injuries with local delivery of neutralising antibody to the pro-fibrotic growth factor TGF- β 1, this study focuses mainly on the development of a new mathematical model to predict the diffusion of human antibody anti-TGF- β 1 through bioengineered membranes. In order to identify the model parameters, laboratory experiments have been set up, characterising the uptake and release of anti-TGF- β 1 to and from FN and HA synthetic nerve guidance channels.

METHODS: Different types of experiments were carried out with a standardised commercial fluorescently labelled human IgG preparation, since anti-TGF- β 1 itself is structurally no different from IgG. The tracing of the distribution of labelled IgG through the biomaterial was performed by spectrofluorimetry.

The first kind of experiments were carried out using FN biomaterial as nerve guidance channels of specific dimensions with an empty core space. The FN tubes were filled with a FITC labelled IgG physiologic solution. Different IgG concentrations were used, namely 1 μ g/ml in one set of experiments and 10 μ g/ml in another set. The two different concentrations were chosen as a compromise between the natural concentration of TGF- β in human tissue and the spectrometer sensitiveness. Sampling was performed at different times, pipetting 100 μ l and detecting the IgG concentration by Luminescence Spectrometer.

For the second type of experiments, FN tubes with a FN core were suspended in physiologic solution (PS) in the middle of the diffusion chamber, before having been soaked overnight in a concentrated FITC labelled-IgG solution (50 μ g/ml) prepared in PS. FN tube sampling was performed at 48, 72 and 144 h and after sampling the FN tubes were cut in 1 mm thick slices and fixed in paraformaldehyd (PFA) in which they were stored until the Luminescence Spectrometer detection.

In the last set of experiments the following dry materials were used: (a) HA sheet, (b) holed (90 to 50 μ m diameter) HA sheet, (c) HA tubes + FN, and (d) holed HA tubes + FN cores.

They were previously cut into small pieces and then soaked with the labeled IgG (4 μ g/ml) PBS solution, for 90 hours at room temperature. After soaking, they were transferred in PBS to monitor over time (2 weeks) the Ab release.

In all experiments, diffusion was performed at room temperature.

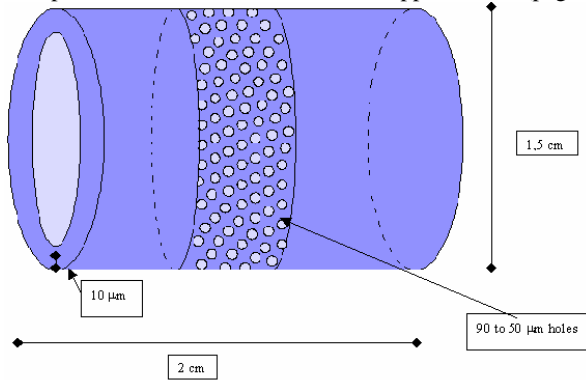


Fig. 1: Scheme of holed HA tube.

RESULTS: In the first set of experiments, results showed that after 3 days, about 15 μg of fluorescent IgG were detectable inside the FN tube, starting from a total of 30 μg , while only 3 μg were detectable outside the tube in the PS surroundings: about 12 μg were missing.

In the second type of experiments the FN tubes reached the maximum of IgG saturation in 72 h. The diffusion rate was very low, reaching in 144 h a maximum of 3.4 % of diffusion.

In the third type of experiments the materials (a) and (b) did not adsorb the Ab, while materials (c), but above all (d) adsorbed the Ab.

DISCUSSION & CONCLUSIONS: The above experimental results, also preliminary, showed that the IgG could be trapped in the FN material for at least the first six days after the implant. According to this, it could be worthwhile to consider the possibility that FN material could be a good Ab depot for long term antibody therapy. In fact, the IgG should stay in the FN material at least 2-4 weeks to get the anti-fibrosis effect during the nerve repair process. The diffusion rate of the first days is critical to the advantage of using therapeutic drugs within the material, in order to retain and release it gradually in vivo.

Based on the promising results obtained, the possible aims for the future could be to improve the detectability of the labelled IgG even at lower concentration in order to mimic as close as possible the natural conditions; and to confirm in longer time experiments, the low diffusion of the human anti-TGF- β 1, instead of a general IgG, through FN tubes. The use of the human anti-TGF- β 1 itself will be crucial, in order to not have cross-reactions of the antibody with FN or other materials. Moreover, the more quantitative, direct measures within this experimental framework by using the human anti-TGF- β 1 antibody will be important to determine its diffusion coefficient (D). This parameter will allow

the mathematical model to give a first level estimation of the retention time of the antibody in the interested bioengineered material.

When all parameters are determined, the modelling environment will allow the surgeons who intend to treat regenerating nerves with such type of antibodies to have reliable estimations of treatment times even for in vivo conditions which can be hardly reproduced in an experimental set up.

Finally, the present results seem to be very promising in regards of the possibility to include several therapeutic factors inside the FN guidance channels in the nerve repair process. In fact, other cytokines have specific roles in nerve regeneration: nerve growth factor (NGF) and fibroblast growth factor (β -FGF) control sensory neuronal survival and out-growth, whereas brain-derived growth factor (BDGF) and ciliary neurotrophic factor (CNTF) control motor neuronal survival and out-growth, and interleukin-1 (IL-1) promotes detersion by scavenger macrophages and increased synthesis of neurotrophic factors [3].

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