

THE EFFECT OF BMP-2 ON LARGE BONE DEFECTS: AN *IN SILICO* STUDY

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Abstract *The healing of large bone defects is a complex process that challenges both tissue engineering and modern orthopaedics. Among all the available strategies that address this issue, the use of bone morphogenetic protein -2 (BMP-2) is becoming more and more popular since BMP-2 has been identified as one of the most powerful osteoinductive proteins. In this work we aim to develop and validate a mechano-chemical regulatory model that predicts in silico the effect of BMP-2 on large bone defect healing.*

1. INTRODUCTION

Bone morphogenetic protein -2 (BMP-2) has captured the attention of many researchers. From the discovery of its remarkable osteoinductive properties in 1965 by Urist, BMP-2 has been identified as a critical element in bone regeneration.

From an *in silico* perspective, large bone defect healing has mainly been addressed considering the use of scaffold constructs. Thus, Adachi et al. [1] developed a computational framework to study the balance between scaffold degradation and new bone formation. Byrne et al. [2] investigated scaffold properties in order to improve bone regeneration. Additionally, Checa and Prendergast [3] combined mechano-regulation with a lattice approach in order to provide a better understanding on scaffold vascularisation and tissue ingrowth.

Previous *in silico* studies focused, for the most part, on the mechanical side of the problem and assessed the scaffolds influence, leaving aside the use of growth factors and drug delivery devices.

In this work, we take a novel approach and propose a novel *in silico* mechano-chemical model, combining both the mechanical stimulus and the chemical stimulus of BMP-2. With this novel model, we aim to predict large bone defect healing, given the effect of

exogenous BMP-2 released inside the defect.

2. METHODS

2.1. Effect of BMP-2 on cells

The development of this model considers the quantitative effect that BMP-2 has on cells. From the literature, it is clear that BMP-2 [4] affects cells in a dose dependent way. From a quantitative perspective, we observed that BMP-2 mostly affects cells proliferation, cell chemotaxis, cartilage hypertrophy and bone tissue production.

The quantitative data on these effects was gathered from the available studies from the literature as shown in Fig. 1. Given the data distribution for each cell behaviour, we proposed adjustment curves that reflect the modulatory effect of BMP-2 on cell behaviour.

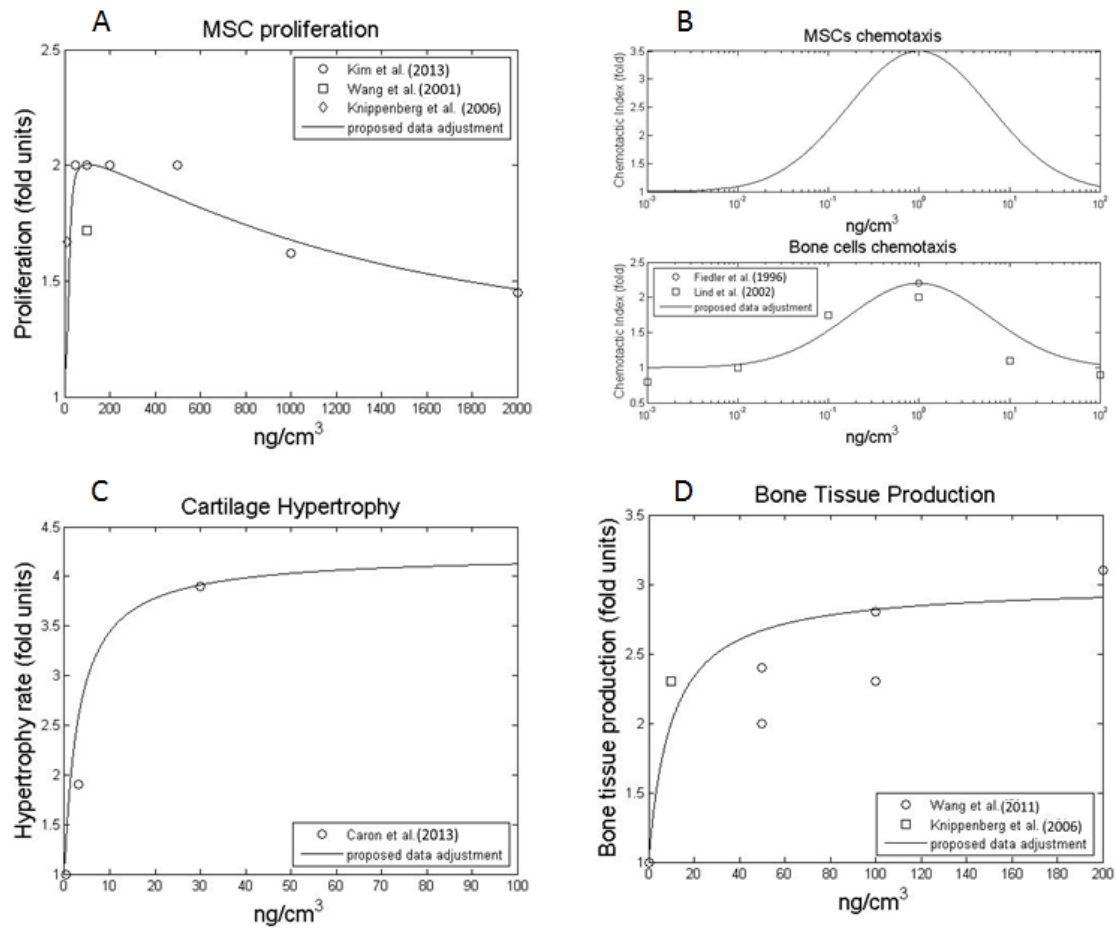


Fig.1 – The effect of BMP-2 on MSCs proliferation (A), chemotaxis of MSCs and bone cells (B), cartilage hypertrophy (C), and bone tissue production rate (D).

2.2. BMP-2 equilibrium

To define the BMP-2 equilibrium equation, we assume that BMP-2 concentration g can

change with: cellular consumption, molecular half-life degradation, cellular production and molecular diffusion. The equilibrium equation therefore stands as

$$\frac{\partial g}{\partial t} = -\left(\frac{\partial g_{events}}{\partial t} + \frac{\partial g_{bkgd}}{\partial t}\right) - \frac{\partial g_{dgrad}}{\partial t} + \frac{\partial g_{prod}}{\partial t} + \frac{\partial g_{diff}}{\partial t} \quad (1)$$

where BMP-2 consumption is divided into background consumption (bkgd) and consumption due to cellular events (events).

Cellular background consumption (bkgd) is defined following Michaelis-Menton kinetics considering BMP-2 as the substrate and BMP-receptors as the catalyst.

As for consumption due to events (events), cell differentiation was considered to be the major event that would bring BMP-2 levels to their physiological minimum. Also, half-life degradation was considered with a half-time (dgrad), with a half-time $t_{1/2} = 0.42days$.

Additionally, BMP-2 was considered to diffuse in the medium (diff) according to Fick's Law. Finally, we assumed that BMP-2 was considered to be produced (prod) mainly by MSCs and bone cells as described in equation.

Given the described effects of BMP-2, these were coupled with the mechanistic model proposed by Gómez-Benito et al. [5] by considering its modulatory effect. The extended model is a mechano-chemical model which considers both the mechanical stimulus proposed by Gómez-Benito et al. [5] and the chemical stimulus of BMP-2 described above.

2.3. BMP-2 in critical size defects

In a recent *in vivo* study by Boerckel et al. [4], a rat femur with an 8mm defect is implanted with a hydrogel soaked with BMP-2. The author experimented with different BMP-2 doses (0µg, 0.1µg, 0.5µg, 1µg, 2.5µg, 5µg) and after 12 weeks concluded that the amount of bone tissue formed inside the 8mm defect is dose dependent.

In order to validate the proposed model, we reproduce *in silico* the experiment performed *in vivo* by Boerckel et al. [4]. We considered the 2D geometrical model presented in Fig. 2 which represents a longitudinal section of the the mouse tibia. Analogously to Boerckel et al. [4] experiments, the gap was filled with alginate hydrogel soaked with different doses of BMP-2 (0µg, 0.1µg, 0.5µg, 1µg, 2.5µg, 5µg).

Moreover, we also tested the model leaving the bone defect as-is. This is an *in silico* control experiment used to verify that the model did not fill the defect when no hydrogel is placed.

3. RESULTS

The results obtained are presented in Fig.3 and Fig.4 and show the bone predicted in the defect after a 12 week period.

Qualitatively, we observe in Fig.3 the distribution of newly formed woven bone formed inside the defect for the different cases simulated. We notice that bone tissue only appears when both the hydrogel and BMP-2 are used. When no hydrogel nor BMP-2 are inserted in the defect, bone is not formed.

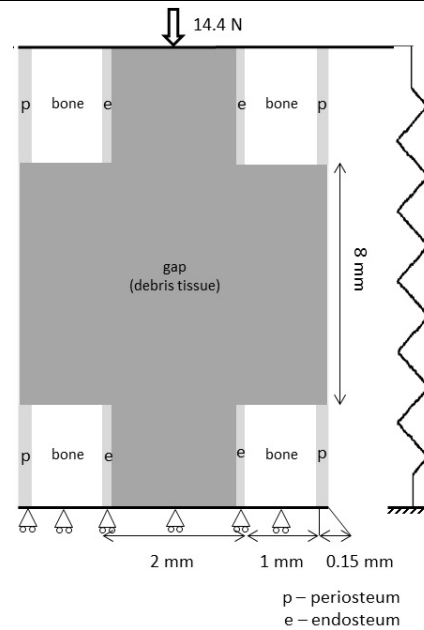


Fig. 2 2D geometrical model used to represent an idealized bone section. The system was mechanically supported by a fixator with rigidity $k=277\text{N/mm}$ as performed by Boerckel et al. [4]

Moreover, it is also clear from Fig.3 that a higher dose of BMP-2 promotes a higher defect infill with bone. Thus, the amount of bone predicted is dose-dependent.

From a quantitative perspective, Fig.4 compares the amount of normalized bone tissue measured *in vivo* with the amount predicted *in silico*. As we can observe the bone amount predicted is very close to the amount of bone measured.

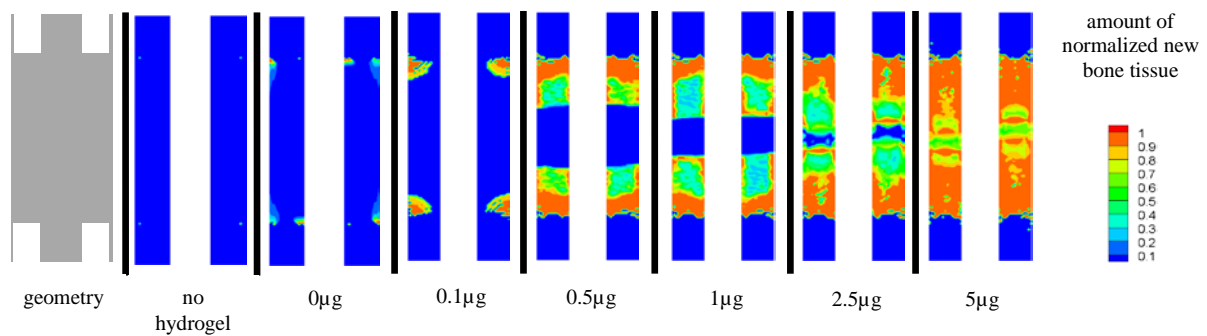


Fig. 3 Normalized amount of bone tissue after 12 weeks for different conditions: no-hydrogel case and hydrogel soaked with different BMP-2 doses ($0\mu\text{g}$, $0.1\mu\text{g}$, $0.5\mu\text{g}$, $1\mu\text{g}$, $2.5\mu\text{g}$, $5\mu\text{g}$)

In Fig.4 the worst prediction occurs for the $1\mu\text{g}$ dose which underpredicts by 10% the *in vivo* measurements. Nevertheless, non-bridging of the defect is still predicted for this dose. For higher doses, when bridging occurs ($2.5\mu\text{g}$ and $5\mu\text{g}$ cases) the *in silico* predictions are very close to the *in vivo* measurements (0.8% and 5.5% errors respectively).

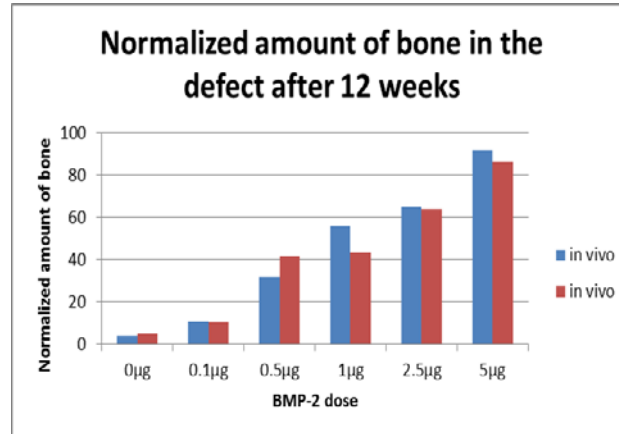


Fig.4 Normalized amount of bone tissue in the defect after 12 weeks for different BMP-2 doses

4. DISCUSSION

The proposed mechano-chemical model incorporates the effect of BMP-2 on cell behavior. Specifically, we incorporated in the model proposed by Gómez-Benito et al. [5] the BMP-2 influence on cell proliferation, chemotaxis, cartilage hypertrophy and bone tissue production. From the results obtained, the model recovered the dose dependent behavior observed *in vivo* [5] as well as the bone amount measured *in vivo*.

Comparing the no-hydrogel case, the case with hydrogel but not BMP-2, and the remaining cases where BMP-2 and the hydrogel are both used, it seems evident that a combination of both the hydrogel and BMP-2 is necessary for bone infill to occur. This happens because the hydrogel is required to provide a physical support for cells to migrate while BMP-2 provides a strong stimulus that attracts cells into the defects. Without such a combination, cells do not invade the defect.

Moreover, from a quantitative perspective, the *in silico* predictions are somewhat accurate: the worst prediction diverges from the *in vivo* results by only 10%. Moreover, given that for lower doses underpredictions can occur, (1µg dose) and that for higher doses where bridging occurs the model predictions were more accurate (2.5µg and 5µg doses), we can say that the model seems to provide a conservative prediction.

From the proposed model it is clear that high doses of BMP-2 are required for bone bridging to take place.

Yet, the proposed model presents some limitations. Firstly the model does not consider the surrounding soft tissues which does not allow ectopic bone to be predicted *in silico*.

Secondly, we use a 2D geometry which, although simple to use, allow for a limited validation only. Contrarily to a 3D model, a 2D model does not allow certain metrics, such as torsion to failure, to be computed in order to provide further validation.

Conversely to previous qualitative models, in the proposed mechano-chemical model we use a quantitative approach. However we are always dependent on the data available in the literature which is not as abundant as one would wish for. In fact, except for the proliferation curves,

we had to assume asymptotic modulatory behavior for higher doses when data was not available (Fig.1). This happened in particular for cartilage hypertrophy and bone productions curves where an asymptotic behavior was considered for higher concentrations. However, whenever more quantitative data come available, it is relatively easy to update the model by updating the modulatory curves (Fig.1).

Finally, BMP-2 is just one in a myriad of factors that take part in bone healing. Our work focused on this factor since it is one of the most important in bone tissue engineering field. Despite that, other factors can also be incorporated in the model, using the same quantitative approach.

5. CONCLUSION

In this work, we proposed a novel *in silico* mechano-chemical approach that puts that emphasizes on the chemical stimulus provided by BMP-2. With this model we successfully predicted defect healing when BMP-2 is delivered by an alginate hydrogel. The qualitative and quantitative agreement obtained suggests that this novel *in silico* tool can provide further insight for bone tissue regeneration strategies such as optimizing the doses of BMP-2, thus enhancing healing outcome.

7. ACKNOWLEDGEMENTS

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