Transforming Growth Factor-beta2 Interaction with Plasma Sprayed Hydroxyapitite: Binding and Release Characteristics at different pH

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Statement of Purpose: Joint replacement with prosthetic implants is a common surgical procedure where success is determined by full integration of the implant with the surrounding bone. Recently, this osteointegration has been enhanced through the use of osteoconductive materials, like hydroxyapatite (HA), delivering osteoinductive agents, like growth factors, to the site of bone regeneration. Local pH at the site of repair may deviate considerably from the physiological levels thus altering the bio-availability of the applied factor due to release kinetics. In this study we investigated the binding and release characteristics of transforming growth factor-beta 2 (TGF-β_2) to plasma flame sprayed HA on titanium (Ti) implants by varying the pH of loading and release buffers in order to elucidate the interaction between the two materials.

Methods: Grit blasted Ti tubes (10mm long, 1.5mm external diameter) were plasma sprayed (gift from Zimmer) to give a coating (~50µm) which is composed of HA (80%), tricalcium phosphate (15%) and uncharacterized calcium phosphate (5%) [1]. All implants for the study were procured from the same batch to minimize variability. TGF-β_2 (gift from Genzyme Biosurgery) was diluted in buffer (30mM citrate/3% mannitol) of pH 2.4, 7.4 or 10.4 to give a final concentration of 63.8µg/ml. Four implants for each condition were loaded with 250ng TGF-β_2 by applying 3.92µl of the solution evenly to the surface. Implants were allowed to air dry completely and stored overnight at 4ºC before use. Release studies were carried out at 37ºC with continuous rotation in release buffer (1% BSA in PBS; 200µl) at pH 3, 4.5, 6, 7.4 and 9. After the initial release for 6 hrs, the buffer was changed and the release was allowed to proceed for a further 18hrs to yield samples for 24hrs. The released TGF-β_2 was quantified with specific ELISA (R&D Systems). All data are presented as % release of the original loading and analyzed by ANOVA.

Results/Discussion: On average approximately 23% of the total loaded TGF-β_2 was released in 24hrs with significantly higher release at 6hrs (21.2%) compared to 24hrs (2.1%; $p<0.0001$). When we analyzed the effect of varying the release pH for a given loading condition, there were distinct patterns of release after 6hrs for each of the loading buffer pH (Figure 1). These patterns were significant for loading pH2.4 and pH7.4 ($p<0.05$) but not for pH10.4. In general, the release was greater at more basic pH. No significance was seen for release at 24hrs time point. This finding indicates that the initial bio-availability due to release of TGF-β_2 from HA at the site of delivery is determined by the local pH. Conversely, when the effect of loading pH for a given release pH was analyzed at 6hrs, overall significance was observed for the release buffer pH3 ($p<0.05$), pH4.5 ($p<0.0005$) and pH6 ($p<0.005$) but not for pH7.4 or pH10.4 (Figure 2). For 24hrs time point, significance was only observed for release at pH6. This data shows that the release of TGF-β_2 from HA at more acidic conditions is determined by the pH of the loading buffer. This study shows that a significant amount of TGF-β_2 is released from the HA at early time point (6hrs). This observation is consistent with our previous findings that majority of the release occurs within the first 24 to 48 hours [2]. Interestingly, our observation that greater release occurs in a more basic release buffer is in disagreement with the study of Matsumoto showing higher release at acidic pH [3]. This discrepancy may be the result of differences in the protein used (cytochrome c, PI ~10.2 vs. TGF-β_2, PI ~8.5) and method of HA preparation (slurry vs. plasma spray).

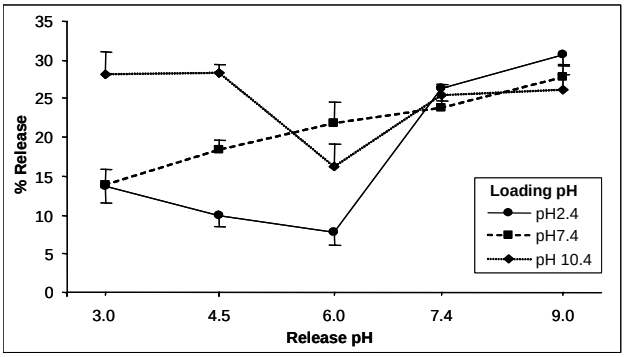

Figure 1. TGF-β_2 release at 6hrs by varying release pH.

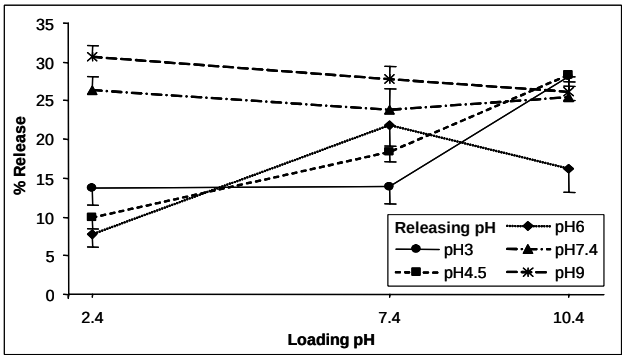

Figure 2. TGF-β_2 release at 6hrs by varying loading pH.

Conclusions: Bio-availability of locally delivered proteins on HA at the repair site can be controlled by defining specific loading pH conditions of the protein.

References: [1] Sumner DR. J Bone J Surg. 1995:77A: 1135-1147. [2] Sumner DR. J Orthop Res. 2001:19:85-94. [3] Matsumoto T. Biomaterials. 2004:25:3807–3812.

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