The Effects Of Hyaluronic Acid And Poly-d,l-lactic Acid Coatings On Titanium Implant Fixation In Sheep

Christina M. Andreasen¹, Susan S. Henriksen¹, Ming Ding¹, Thomas Levin Andersen², Soeren Overgaard¹. ¹Odense University Hospital, University of Southern Denmark, Odense, Denmark, ²Vejle Hospital-Lillebaelt Hospital, University of Southern Denmark, Odense, Denmark.

Disclosures:  C.M. Andreasen: None. S. S. Henriksen: None. M. Ding: None. T. Levin Andersen: None. S. Overgaard: None.

Introduction: Due to its high biocompatibility autogenous bone has for decades been considered the gold standard in joint arthroplasty. However, the use of autograft is associated with increased donor site morbidity and limited availability. Likewise, with a risk of disease transmission and lack of osteogenic potential, allogenic bone as filling material also exhibits some major disadvantages. Consequently, several synthetic alternative bone materials have been considered for treatment of bone defects, e.g. hydroxyapatite (HA) combined with tricalciumphosphate (TCP) is often used. It is postulated that infiltration of HA/β-TCP scaffold with the polymer poly (D, L)-lactic acid (PDLLA) is expected to increase its mechanical properties, and might further enhance implant fixation. Likewise, the polysaccharide hyaluronic acid (HyA), shown to stimulate the bone forming osteoblasts, is expected to increase the bone formation efficacy of a synthetic scaffold material. The objective of this experimental study was to investigate the osseointegrative effect of HA/β-TCP-PDLLA, HA/β-TCP-HyA and pure HA/β-TCP versus allograft on implant fixation in a critical size defect model in sheep. We hypothesized that the effects of adding PDLLA and HyA to substitutes on implant fixation were similar to that of allograft, thus might be considered as alternative materials used in implant fixation.

Methods: Cylindrical, plasma-sprayed titanium implants (10 mm in height and 6 mm in diameter, pore size 480 µm) were used. The implant had a footplate and top washer both 10 mm in diameter resulting in a 2 mm circumferential gap with a volume of 0.5 ml. Four titanium implants were inserted extra-articularly into the medial and lateral femoral condyles of eight female adult sheep, resulting in 32 implants in total. The concentric gap was filled with one of the four materials randomly: allograft obtained from a healthy sheep as control, HA/β-TCP, HA/β-TCP-PDLLA or HA/β-TCP-HyA. The basic HA/β-TCP granules consisted of 70% HA and 30% β-TCP. The PDLLA reinforced HA/β-TCP contained 12% organic polymer PDLLA (50% D-PLA, 50% L-PLA, MW = 308 kDa). HA/β-TCP-HyA granules were coated with a HyA solution (0.15% w/w) under sterile conditions. All granules had a porosity of ~80%. Surgical intervention on the femur condyles was performed under sterile conditions by a lateral incision drilling a 10 x 11 mm² (height x depth) cylindrical hole. A titanium implant was inserted into the hole. After filling one of the four materials, a top washer sealed the gap. The same procedure was repeated for the medial side and for the opposite femur. Observation time of 12 weeks was chosen according to our previous studies [1]. The sheep were euthanized 12 weeks after surgery and the femur condyles were harvested. Each bone implant specimen was divided into two parts: one 3.5 mm thick specimen for micro-CT scanning and subsequently mechanical test, and one 5.5 mm thick specimen for histology and histomorphometry. The bone specimens were scanned to quantify the microarchitectural properties of gap mass consisting newly formed bone and substitute. The mechanical test was to assess the shear
mechanical properties of the interface of the newly formed bone surrounding the implant. Histomorphometric analysis was used to quantify the bone formation and tissue ingrowth at the surface of the titanium implant.

**Results:** The microarchitectural data revealed a statistically significant difference in the volume fraction among the four groups. The volume fraction in the allograft group was significantly lower than in the HA/β-TCP and the HA/β-TCP-HyA groups (Figure 1A). Likewise, the trabecular thickness was lower in the allograft group compared to the HA/β-TCP and HA/β-TCP-HyA groups. There was no significant difference among the groups regarding neither the connectivity density nor the trabecular separation. Similar to the volume fraction, apparent and material densities were significantly lower in the allograft group than in the HA/β-TCP-HyA and HA/β-TCP groups. The push-out test revealed no statistically significant difference in the shear mechanical properties among the four groups (Figure 1B).

Histologically, new bone formation was present in all groups with remnants of bone substitute in the three groups added bone substitute (Figure 2A-D). Although new bone formation was the largest in the allograft group, there was no statistically significant difference in the allograft group compared with the HA/β-TCP-HyA and HA/β-TCP groups, whereas the percentage of new bone in the HA/β-TCP-PDLLA was significant lower compared to the allograft group (Figure 1C).

**Discussion:** The current study investigated the effects of adding PDLLA and HyA to HA/β-TCP on the early implant fixation in a 2-mm gap in vivo model in sheep. The significant higher volume fraction and trabecular thickness in HA/β-TCP and HA/β-TCP-HyA compared to allograft is due to non-absorbed remnants of bone substitute, which cannot be distinguished from newly formed bone by micro-CT. This also explains the significant higher apparent and material density in the same groups. Despite a significant difference in the microarchitecture among the four groups, the histological evaluation revealed no significant difference in the percentage of newly formed bone surrounding the implants comparing the allograft group to the HA/β-TCP-HyA and the HA/β-TCP groups. Additionally, there was no difference in the mechanical properties comparing the four groups. This study supported our hypothesis that adding PDLLA and HyA to substitutes on bone formation were similar to allograft. Based on this investigation, it was suggested that HA/β-TCP-PDLLA and HA/β-TCP-HyA on bone formation were at least as good as allograft, and might be considered as alternative substitute materials for bone formation.

**Significance:** The clinical relevance of this study is that adding PDLLA and HyA to substitutes on titanium implant fixation might be a good alternative to allograft with sufficient bone formation and mechanical stability.
Figure 2. Histological sections for four groups showing new bone formation in the concentric gap: I. Implant (black color); NB. New bone (blue color); HB. Host bone (blue color distant from implant); M. Bone marrow (white color); R. Remnants of substitute (Black color, minor areas) (HAβTCP, HAβTCP-HyA, HAβTCP-PDLLA). Allograft (3A), HAβTCP-group (3B). HAβTCP-HyA (3C) and HAβTCP-PDLLA (3D). Newly formed bone was clearly identified together with fibrous tissue and bone marrow in all three groups. Remnants of nonabsorbed substitute were identified in all the groups containing substitute. The sections were stained with light green and basic fuschin; x4 magnification.

ORS 2015 Annual Meeting
Poster No: 0299