In vitro release and antimicrobial activity of gentamicin salts as coating for bone allografts

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INTRODUCTION

Bone grafting is usually required for patients with bone stock loss due to hip or knee reconstruction, in both complicated primary cases as well as revision cases. Regarding the complications related to obtainment of autologous bone grafts, allogeneic bone has been used as natural alternative. However, the rapidly increasing number of joint arthroplasties performed around the world has also seen a rising number of complications. Infections are one of the most important cases. Since the contamination is normally associated to metal surfaces and dead tissues like the bone grafts, local delivery of antibiotics is an option for therapy and prophylaxis of implant associated infections. In this study, we tested gentamicin palmitate (GP) mixed with gentamicin sulfate (GS) as a coating for bone allografts.

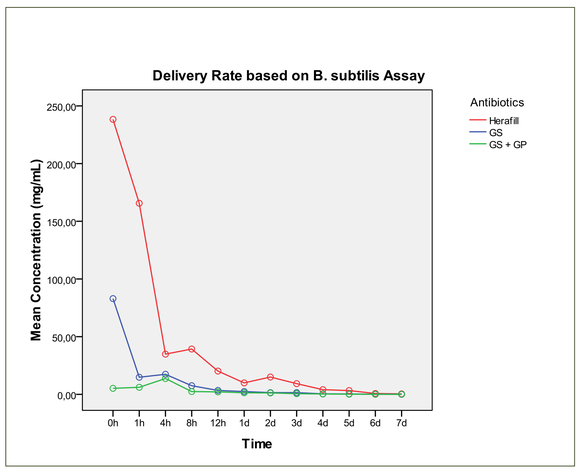
METHODOLOGY

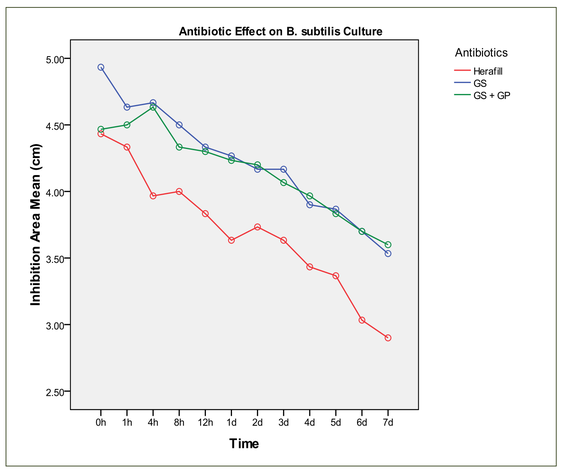
1. Obtainment of bone chips (BCh) by morsellising femur heads in pieces around 5-10 mm using a bone mill;
2. Impregnation of BCh with antibiotic (Table 1);
3. *In vitro* drug release in phosphate-buffered saline (PBS) pH 7.4 at 37°C, over rocking table during 0, 1, 4, 8 and 12 hours and 1, 2, 3, 4, 5, 6 and 7 days;
4. Determination of released concentrations by analysis of agar inhibition area measurement using *Bacillus subtilis;*
5. Inhibition area determination for antimicrobial efficacy using *Staphylococcus aureus* ATCC 29913 *and Staphylococcus epidermidis* ATCC 12228;
6. Statistical analysis using One Way ANOVA and Sheffe/Games-Howel as post hoc.

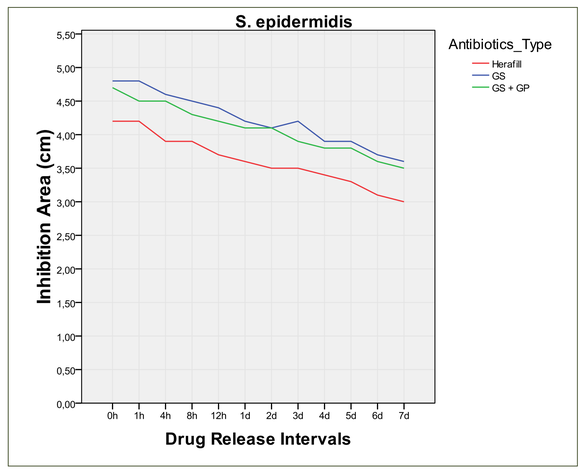
BCh + GS+GP (50:50)	1:1
BCh + GS pure	10:1
BCh + Herafill®	2:1

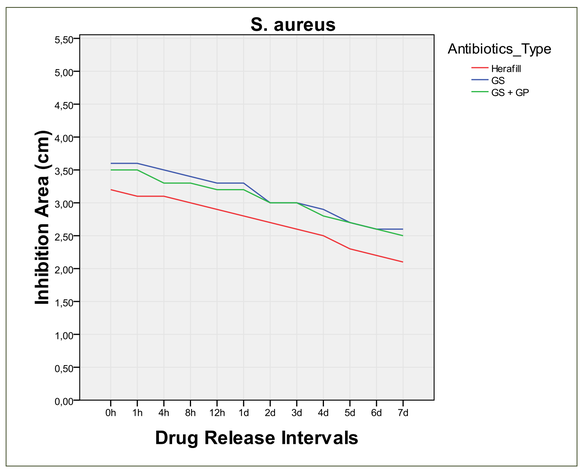
Table 1 - Proportion between antibiotic substances and bone chips for impregnation. Bone chips (BCh), Gentamicin Sulfate (GS) and Gentamicin Palmitate (GP).

RESULTS









	ANTIBIOTIC SUBSTANCES	SIGNIFICANCE (P) BETWEEN CONCENTRATION ALONG TIME											
		0h	1h	4h	8h	12h	1d	2d	3d	4d	5d	6d	7d
B. subtilis	Herafill® vs. GS	0.24	0.10	**0.03**	-	0.47	0.30	0.24	0.11	0.10	0.37	0.58	**0.00**
	Herafill® vs. GS+GP	**0.04**	0.09	**0.01**	**0.00**	**0.03**	0.25	0.23	0.09	0.10	0.27	**0.03**	-
	GS vs. GS+GP	0.23	0.13	0.75	**0.01**	0.42	0.16	0.79	0.10	0.59	0.96	0.12	0.31

	ANTIBIOTIC SUBSTANCES	SIGNIFICANCE (P) BETWEEN INHIBITION AREA ALONG TIME											
		0h	1h	4h	8h	12h	1d	2d	3d	4d	5d	6d	7d
B. subtilis	Herafill® vs. GS	**0.00**	**0.05**	**0.00**	-	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.02**
	Herafill® vs. GS+GP	0.95	0.22	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	-
	GS vs. GS+GP	**0.01**	0.10	0.78	**0.06**	0.86	0.92	0.93	0.51	0.70	0.92	1.00	0.64

	ANTIBIOTIC SUBSTANCES	SIGNIFICANCE (P) BETWEEN ANTIBIOTIC SUBSTANCES ALONG TIME											
		0h	1h	4h	8h	12h	1d	2d	3d	4d	5d	6d	7d
S. epidermidis	Herafill® vs. GS	**.005**	**.000**	.026	**.002**	**.008**	.031	**.000**	**.005**	**.007**	**.000**	**.001**	**.002**
	Herafill® vs. GS+GP	-	**.002**	.024	.011	.036	**.001**	**.000**	.011	.011	**.007**	**.003**	**.006**
	GS vs. GS+GP	.102	.001	.102	.330	.068	.817	.834	.017	.201	.102	.296	.394
S. aureus	Herafill® vs. GS	**.000**	-	**.001**	**.000**	**.000**	**.000**	.068	.011	.011	**.003**	.010	**.003**
	Herafill® vs. GS+GP	**.008**	.015	.013	**.000**	**.002**	**.002**	.028	**.002**	.018	**.004**	**.005**	**.008**
	GS vs. GS+GP	.016	.102	.088	.037	.187	.125	.680	.645	.201	.900	.751	.512

• Taking into consideration that a standard-length hip reconstruction typically requires around 100 grams of bone chips, once impregnated with GS+GP at the same concentrations used in this study, the estimated amount of drug delivered locally would be 600 mg/mL after 24 hours and 3 mg/mL after 7 days. The bone grafts impregnated with GS pure could reach a local delivery rate of 2500mg/mL after 24 hours to 7 mg/mL after 7 days.

• A similar behavior of the different substances against *Staphylococcus aureus* and *Staphylococcus epidermidis* was observed. For both strains GS+GP and GP pure were more effective than Herafill®. *S. epidermidis* is significantly more susceptible to Herafill®, GS+GP and GP pure than *S. aureus*.

CONCLUSION

The capacity of bone grafts to act as antibiotic carrier has been confirmed in this study. GS+GP showed equivalent efficacy against *S. aureus* and *S. epidermidis* compared with GS pure. The lower delivery rate of GS+GP, related to its affinity with fat tissue can be an advantage for longer release time increasing the local protection against infections.

This study was sponsored by Heraeus Medical GmbH.